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SUBVERSION OF INNATE IMMUNE SIGNALS BY *SCHISTOSOMA MANSONI* PERMITS WORM DEVELOPMENT

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Helminth blood flukes of the genus *Schistosoma* infect over 200 million people world wide. Perhaps as a result of extensive host-parasite co-evolution, *S. mansoni* has evolved to exploit host immune factors as signals to coordinate its own development within the host. Worms fail to develop normally in $RAG^{-/-}$ mice that lack all T and B cells, while development is restored when $CD4^+$ T cells are transferred into $RAG^{-/-}$ mice, suggesting that $CD4^+$ T cells play a central role in regulating parasite development. However, recent findings suggest the role of $CD4^+$ T cells in this process is indirect, being limited to provision of non-cognate T cell help for innate responses which, in turn, facilitate parasite development. In support of this hypothesis, we have found that administration of LPS to $RAG^{-/-}$ mice, in the absence of $CD4^+$ T cells, also restores worm development, indicating that innate immune signals are sufficient for parasite development to proceed normally. LPS, a pathogen-associated molecular pattern (PAMP), activates TLR-4, resulting in signaling through both MyD88-dependent and TRIF-dependent pathways. Interestingly, specific stimulation of TRIF signaling using monophosphoryl lipid A (MPLA) or poly-IC failed to restore worm development in $RAG^{-/-}$ mice, as did administration of exogenous IFN-α, suggesting that worm development is not dependent on TRIF-mediated induction of type I interferon expression. Our current research efforts are therefore focused on dissecting the MyD88-linked signaling events that influence schistosome development. Elucidation of the innate immune signals that control schistosome development could provide leads for the development of new drug targets and vaccine strategies.

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REGULATION OF INNATE IMMUNITY TO LEISHMANIA INFECTION BY TYPE I IFN SIGNALING

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Type I IFNs exert diverse effector/regulatory functions in innate and adaptive immune responses to viral and non-viral infections; however, their roles in parasitic infections are less clear. In murine models of *Leishmania* infection, it has been reported that parasite-induced type I IFNs are critical for NO-dependent disease control, and that administration of IFN-β has a dose-dependent protective effect in *L. major*-infected mice. Surprisingly, we found that compared to WT controls, IFNAR-/- mice developed significantly smaller lesions and reduced Ag-specific immune responses following infection with *L. amazonensis* (*La*). The marked reduction in tissue parasite loads even at 3 days of infection in IFNAR-/- mice suggested the possibility of neutrophil-mediated parasite clearance. This hypothesis was supported by *in vitro* and *in vivo* studies. First, IFNAR-/- mice showed sustained infiltration of neutrophils, but limited recruitment of CD11b+Ly6C+ inflammatory monocytes, in inflamed ear tissues at day 7 and in inflamed peritoneal cavity at day 2. Second, while macrophages responded comparably to parasites, the interactions between macrophages and IFNAR-/- neutrophils (but not WT or STAT1-/- neutrophils) greatly enhanced parasite killing. Third, in comparison to WT and STAT1-/- counterparts, IFNAR-/- neutrophils had significantly higher rates of spontaneous and infection-induced apoptosis and released higher levels of neutrophil elastase and myeloperoxidase. Finally, while co-injection of IFNAR-/- neutrophils with parasites reduced parasite survival, co-injection of WT neutrophils with parasites or adoptively transfer of WT neutrophils into IFNAR-/- mice markedly enhanced tissue parasite loads. This study indicates an important role for type I IFNs in regulating neutrophil turnover and *Leishmania* infection and provides new information on innate immunity to protozoan parasites.

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VIRAL DETERMINANTS OF DENGUE VIRUS FITNESS AND VIRULENCE REVEALED IN THE EVOLUTION OF DENGUE VIRUS SEROTYPE 2 IN NICARAGUA

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Four serotypes of dengue virus (DENV-1-4) circulate globally, causing more human illness than any other arbovirus. DENV infection results in dengue fever, a debilitating acute febrile illness, or the more severe, life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The genetic make-up of DENV, in addition to host immune status and genetic variables, contributes to disease severity. Viral evolution may result in increased DENV replication in host cells or altered sensitivity to neutralization or enhancement by host antibody responses. Here we describe an increase in dengue disease severity between 2005 and 2006-7 in two independent studies in Managua, Nicaragua, that cannot be explained by clinical/epidemiological variables or by introduction of a new DENV serotype or strain. Whole-genome sequencing of viruses isolated from patient sera reveals the evolution of a new clade of Asian-American genotype DENV-2 between 2005 and 2006-7 in Nicaragua ($p<0.0001$). Consistent with a model of replacement of the circulating DENV-2 clade by a more fit and perhaps more virulent clade, the new DENV-2 clade correlates with DHF/DSS ($p<0.05$). The hypothesis of increased fitness/virulence is being tested *in vitro* by infection of cell lines and primary cells of monocytic lineage with primary viral isolates and infectious clones representing both Nicaraguan DENV-2 clades. Infection assays are being performed independent of Ab-mediated entry in both U937/DC-SIGN and primary human dendritic cells. To ask if increased fitness occurs in the context of secondary infection, infectivity of viruses from both clades is being tested in THP-1 cells through Ab-mediated infection. Further, the relative fitness of both viral clades is being examined in the context of the host immune response by measuring the relative neutralization of the two clades by pre-existing heterotypic antibodies using patient sera from the Nicaraguan children from whom the viruses in question were isolated. Mutations identified through phylogenetic analyses, including several changes in E, NS1 and NS5 proteins as well as in the 5′ and 3′ UTRs are being assayed for effects on viral replication. The observed increase in disease severity associated with evolution within a single genotype of DENV-2 over a limited time span and geographic range provides a unique opportunity to explore the viral determinants of DENV fitness and virulence.

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ASSESSING THE ROLE OF HUMAN MOVEMENT IN THE TRANSMISSION DYNAMICS OF DENGUE VIRUS IN IQUITOS, PERU

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Entomological risk of infection with dengue virus is usually evaluated at the household level, even though individual human hosts move frequently during the day when the vector, *Aedes aegypti*, is active. With a simple model we illustrate that variation in exposure to infective mosquitoes due

to movement among sites constituting their 'activity space'--i.e. school, work, relative's homes--potentially has a large effect on individual risk and virus transmission. To test this hypothesis, we conducted semi-structured interviews on febrile patients detected by community-based surveillance to identify locations visited in the previous 15 days in the Amazonian city of Iquitos, Peru. Potential human contacts were recruited from residential sites identified in interviews to participate in the study. Blood samples were taken at day 0 and day 15 from both index and contact cases and tested for dengue virus infection by RT-PCR or for dengue-specific antibody by IgM-ELISA. Between August of 2008 and February of 2009, we conducted 16 dengue positive clusters (261 contacts) and 4 dengue negative clusters (71 contacts). Index cases visited between 1 and 8 locations outside the home (mean = 3 sites). Thirty-one percent of participants (86/277) in dengue-positive clusters had laboratory evidence of dengue infection versus 20% of participants (15/75) in dengue-negative clusters (p = 0.06) during a virgin soil outbreak of DEN4. Of 54 sites associated with positive clusters, 25 (46%) had at least one dengue infection compared to 3 of 13 (23%) sites in negative clusters. Total female mosquitoes collected from sites within positive clusters was significantly greater than negative clusters (p = 0.014). These results support the hypothesis that risk of dengue virus infection is associated with exposure to mosquitoes across an individual's activity space and implicate human movement as a key behavioral component influencing pathogen transmission.

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AGE SHIFTS OF DHF IN BRAZIL: INSIGHT FROM A SEROLOGICAL SURVEY IN RECIFE

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Dengue was reintroduced into Brazil in 1986. During the 2007 dengue epidemic, the number of dengue hemorrhagic fever cases more than doubled and a shift in the age distribution was reported. While previously, the majority of DHF cases occurred among adults, in 2007, 53% of cases occurred in children under 15 years old. The shift was pronounced in the north east of the country. The reasons for this shift have not been elucidated. Here, we consider a gradual shift in the age distribution of immunity since the re-emergence of dengue as a possible cause of this shift. An age stratified cross-sectional seroepidemiologic survey was conducted in Recife, Brazil in 2006 in three areas of distinct socio-economic, and infrastructural conditions. Serostatus was determined by ELISA using the kit PANBIO Dengue IgG. Analysis: We used a modified version of the model described previously, to estimate time-constant and time-varying forces or infection of DEN between 1986 and 2001. We used discrete time simulation to estimate the accumulation of monotypic and multitypic immunity over time in a population previously completely susceptible to DEN. Results: The dataset includes data on 1427 subjects aged 5-20 years. The overall prevalence of DEN IgG was 0.80. The time-constant force of infection for the period was 0.052 (95% CI 0-0.17). Simulations show that as time since re-emergence of dengue goes by, multitypic immunity accumulates in adults while monotypic immunity remains in younger age groups. For force of infection of 0.07, the median age of those monotypically immune can be expected to shift from 25 years, 10 years after introduction, to 11 years at equilibrium. Of those monotypically immune, the proportion under 15 years old shifts from 12% to 72%. Higher forces of infection are associated with smaller median and modal ages of monotipycally immune. In conclusion, assuming that persons who have been monotypically exposed are at highest risk for DHF, the shift towards younger ages of that is being observed in Brazil can be partially explained by the accumulation of multitypic immunity in older age groups, 22 years after the re-introduction of Dengue. Heterogeneities in age distribution of DHF cases across the country may be a result of heterogeneous forces of infection in different settings. Serotype specific seroepidemiologic studies are necessary to accurately estimate the serotype specific forces of infection.

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WHAT IS ENHANCED SURVEILLANCE? DESCRIPTION OF AN ENHANCE DENGUE SURVEILLANCE SYSTEM MODEL - THE PATILLAS ENHANCED DENGUE SURVEILLANCE SYSTEM (PEDSS), PATILLAS PUERTO RICO

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Passive laboratory-based surveillance is used to monitor dengue in Puerto Rico and has several limitations; e.g., under reporting, incomplete data, high level of indeterminate specimens (*i.e.*, negative results in the sole acute phase specimen). To address these limitations, an Enhanced Dengue Surveillance model was developed in Patillas, Puerto Rico (P-EDSS). This paper describes the performance after two years of enhanced passive dengue surveillance implementation. P-EDSS was developed at the principal municipality health center. On-site CDC personnel implemented enhanced surveillance procedures, consisting of: 1.) clinician training, 2.) review and correction of each dengue case reporting form using medical chart information, 3.) reminders encouraging patients to return for convalescent sample collection, 4.) monthly performance review meetings. Performance indicators were measured and monitored, and included: reporting rate of suspected cases (i.e., patients with dengue-like illness for which sera was submitted for testing); rate of laboratory-positives (i.e., cases with virus detected by RT-PCR or IgM positivity in any sample); and proportion of indeterminates. When compared to passive surveillance in Patillas in years prior to P-EDSS, we noted up to a 19-fold increase in the rate of suspected cases and up to a 5-fold increase rate in laboratory-positive cases. When compared to neighboring municipalities during the same period, we found up to an 85-fold higher rate of suspected cases and up to a 23-fold higher rate of laboratory-positive cases in Patillas. After the second year, the proportion of indeterminates from Patillas decreased significantly from 59.0% to 46.3% ($p<0.005$). In conclusion, substantially higher levels of suspected case reporting and detection of laboratory-positives, and significantly lower levels of incomplete laboratory evaluations were achieved. P-EDSS allows for increase confidence in the completeness and representativeness of the surveillance data. P-EDSS provides better population-based estimates than passive dengue surveillance.

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TUMOR NECROSIS FACTOR (TNF) AND LYMPHOTOXIN-A (LTA) GENE ASSOCIATIONS WITH DENGUE VIRUS INFECTION IN ETHNIC THAIS

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TNF and LTA are important vasoactive modulators of the immune response and are up-regulated in dengue virus (DENV) infections. Both TNF and LTA are encoded by adjacent gene loci in the central or class III region of the human major histocompatibility complex (MHC) in between class I and II human leukocyte antigen (HLA) genes. A variety of single nucleotide

polymorphisms (SNP) have been identified in non-coding promoter-like regions, adjacent to exons encoding TNF and LTA. Linkage disequilibrium (LD) between TNF, LTA and HLA class I and II genes contributes to the formation of haplotypes or stable combinations of SNP-defined alleles that vary in composition and frequency both within and between different ethnic groups. We have analyzed a variety of SNPs in the TNF and LTA genes of 435 ethnic Thais with subclinical DENV infection, primary and secondary dengue fever (DF), and dengue hemorrhagic fever (DHF). The TNF -238A polymorphism marking the TNF-4,LTA-3 haplotype, was significantly increased in patients with secondary DHF (15.2%) compared to secondary DF (4.1%) (P= 0.0009, Pc=0.022; OR = 4.13). In a subset of patients the LTA-3 haplotype associated with *in vivo* intra-cellular production of LTA and TNF. Furthermore, two extended haplotypes containing TNF-4 and LTA-3, together with HLA-B48, B57 and DPB1*0501, were detected only in patients with secondary DHF. In summary, our analysis has revealed TNF-specific SNPs that significantly associate with DHF in patients with secondary infections. We have evidence that certain LTA haplotypes associate with *in vivo* LTA and TNF protein production, as measured during the acute viremic phase of DENV infection. We have also identified TNF,LTA haplotypes that associate uniquely with secondary DHF and contain relatively rare HLA class I alleles, as well as null expression variants and deletions of other MHC loci. These observations suggest that a variety of gene products encoded within the human MHC act together in determining disease outcome in DENV-primed individuals undergoing secondary infections.

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ROLE OF B CELL MEMORY IMMUNITY IN SECONDARY DENGUE VIRUS INFECTIONS IN MICE

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Dengue, caused by four dengue virus serotypes (DENV-1-4), is the most prevalent mosquito-borne viral disease in humans. The immune response to one serotype plays a role in both protection and pathogenesis of a secondary (2°) DENV infection with a distinct serotype. While adaptive T cell immune responses have been shown to protect as well as enhance 2° DENV infections, little mechanistic data exist about the memory B cell response. We recently demonstrated antibody (Ab)-dependent enhancement (ADE) of DENV infection and disease in a passive transfer model in interferon-α/β and -γ receptor-deficient mice (AG129), in which mice die of a vascular leak syndrome at day 4-5 post-infection (p.i.). We have also shown that DENV-infected AG129 mice develop and maintain neutralizing Abs up to 20 weeks p.i. and that the Ab isotype composition is relatively balanced (IgG1:IgG2a:IgG2b = 1:4:1). In contrast to the passive transfer model, mice infected with DENV-1 or DENV-4 followed 8 weeks later by 2° infection were protected against both a sublethal (10^5 PFU) or lethal (10^7 PFU) DENV-2 D2S10 infection. Cyclophosphamide (CP) is an alkylating agent that inhibits cell proliferation and suppresses the cellular immune response; however, terminally differentiated long-lived plasma cells (LPC) are not affected by CP treatment. The levels of pre-formed anti-DENV Abs were not altered by CP treatment of immune mice. Immune mice treated with CP prior to 2° infection succumbed on day 7.5-8.5, demonstrating that the cellular memory immune response is necessary for protection against DENV infection. However, CP-treated mice died 3-4 days later than naïve CP-treated mice experiencing a primary infection with DENV-2 D2S10, indicating that pre-formed Abs and LPC contribute to protection but are not sufficient to achieve full protection. The survival data correlated with increased viremia in CP-treated mice late in infection. Numbers of splenic memory B cells increased at day 3 post-2° infection. This correlated with increased expression of the proliferation marker Ki67, demonstrating that memory B cells proliferate after DENV 2° infection. We are currently characterizing the antigen-specific memory B cell response and dissecting the role of each adaptive immune subset in 2° DENV infection, focusing on B cells and B cell epitopes. Understanding B cell memory responses in protection versus enhancement will be useful for dengue vaccine development and evaluation.

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HUMANIZED MICE SHOW DIFFERENCES IN DISEASE PRESENTATION ACCORDING TO INFECTING DENGUE VIRUS GENOTYPE

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Humanized NOD-*scid IL2rγ* ^{null} mice infected with 8 different strains (representing the four genotypes) of dengue serotype 2 (DEN-2) virus present with clinical signs of human-like disease, including fever, viremia, erythema and thrombocytopenia. "Humanized" mice are transplanted with human cord blood-derived hematopoietic progenitor cells ($CD34^+$) and develop high levels of human lymphocytes ($CD45^+$ cells) in their peripheral blood; in this study, rates of reconstitution ranged from 16 to 80% (median: 52%). Subcutaneous infection (with approximately 10^6 PFU of each strain, the equivalent of a mosquito bite) of these mice produced a high viremia extending to days 12-18 post-infection. There was a significant decrease in platelets at day 10 in most of the mice and an increase in body temperature (fever) and erythema (rash) in comparison with humanized mice inoculated with cell culture media only. Comparison of Southeast Asian (SE Asian) and other genotype viruses (American, Indian, West African) in this model showed significant differences in magnitude and duration of viremia and rash, with the SE Asian viruses always highest. Indian genotype viruses produced lower viremias and less thrombocytopenia, while West African genotype viruses produced the shortest periods of viremia and the lowest rash measurements. These results correlate with virulence and transmission differences described previously in primary human target cells and whole mosquitoes and correlate with epidemiologic observations around the world. These characteristics make this mouse model ideal for the study of dengue pathogenesis and the evaluation of vaccines and antivirals.

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A LARGE SCALE LABORATORY INVESTIGATION OF THE RELEASE OF INSECTS CARRYING A DOMINANT LETHAL GENE (RIDL®) AS AN EFFECTIVE CONTROL STRATEGY FOR *AEDES AEGYPTI* MOSQUITOES

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The release of insects carrying a dominant lethal (RIDL®) is an attractive novel strategy for the suppression of *Aedes aegypti*, particularly when the lethal trait is repressible, late-acting, and female-specific. Novel methods such as these have become increasingly important due to the expansion of the geographical area in which Dengue viruses (DENV1-4) are endemic. RIDL can be considered an expansion of the sterile insect technique (SIT) and previous mathematical models incorporating both indicate that RIDL may prove more effective than SIT in reducing *Ae. aegypti* populations and thus dengue transmission; namely, because 'RIDL males', not having undergone radiation, should compete more effectively with wild type males and because late- rather than early-acting lethality allows for the effects of density-dependent larval mortality. This is the first study to directly assess the efficacy of the RIDL strategy in suppressing or eradicating *Ae. aegypti* populations using a dominant lethal gene that results in flightless females. This study was conducted as a prelude to field greenhouse trials in Tapachula, Mexico. We will report on large (>300 ft^3) laboratory cage experiments in which males homozygous for the lethal trait will be released into established wild type populations (wt) at either one of two release ratios (10:1 or 5:1 RIDL:wt). We aim to monitor changes in *Ae. aegypti* population size among the treatment and

control groups over time using weekly assessments of egg, pupal, and adult densities. In addition, subsets of the larval populations from each treatment group will be periodically screened to determine the prevalence of the lethal allele in these populations over time. The implications for potential field use of this strain and genetic control strategy will be discussed.

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GENETIC CONTROL OF *AEDES* MOSQUITOES TO PREVENT DENGUE AND CHIKUNGUNYA

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In the absence of vaccines, control of dengue and chikungunya can only be achieved by suppression of mosquito populations. However, current control methods are inadequate to reduce the population of *Aedes* mosquitoes below the disease transmission threshold. Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes. The genetic approach that is closest to practical application is "population suppression" based on the Sterile Insect Technique (SIT). SIT has been used successfully for the suppression or local elimination of several insect species in agriculture, and mathematical modeling indicates that it would be effective against *Aedes* mosquitoes. Sterile male mosquitoes are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Sterility has conventionally been induced with γ-irradiation, which is too damaging for most mosquitoes, or chemicals which are no longer approved. In the RIDL® method mosquito strains are homozygous for one or more dominant lethal genes. We have successfully constructed RIDL strains of *Aedes aegypti* and *Aedes albopictus*, using the tetracycline-repressible 'tet-off' gene expression system to repress the lethal effect with dietary tetracycline. The first RIDL strains have been successfully tested in confined semi-field conditions for mating competitiveness with wild-type mosquitoes and a range of life history and behavioural traits. An area-wide control program based on mass-release of mosquitoes would preferably not release biting females. Sex-separation methods are therefore required. Effective mechanical separation methods are available, and we are developing genetic sexing methods which will be more accurate and efficient. Our successful development of such systems in *Aedes aegypti* will be discussed.

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THE EFFECT OF GENE DRIVE ON CONTAINMENT OF TRANSGENIC MOSQUITOES

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Mosquito-borne diseases such as malaria and dengue fever continue to be a major health problem through much of the world. Several new potential approaches to disease control utilize gene drive to spread anti-pathogen genes into the mosquito population. Prior to a release, these projects will require trials in outdoor cages from which transgenic mosquitoes may escape, albeit in small numbers. Most genes introduced in small numbers are very likely to be lost from the environment; however gene drive mechanisms enhance the invasiveness of introduced genes. Consequently, introduced transgenes may be more likely to persist than ordinary genes following an accidental release. Here, we develop stochastic models to analyze the loss probabilities for several gene drive mechanisms, including homing endonuclease genes, transposable elements, Medea elements, the intracellular bacterium Wolbachia, engineered underdominance genes, and meiotic drive. We find that Medea and Wolbachia present the best compromise between invasiveness and containment for the six gene drive systems currently being considered for the control of mosquito-borne disease.

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GENE EXPRESSION PROFILE ANALYSIS OF *ANOPHELES GAMBIAE* AGING AND BLOOD FEEDING: IDENTIFICATION OF CANDIDATE GENES FOR AGE GRADING

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Information on population age structure of mosquito vectors under natural conditions is not only fundamental to the understanding of vectorial capacity, but it is also crucial to the assessment of the impact of vector control measures on malaria transmission. A recent study demonstrated that transcriptional profiles of the genes strongly associated with age are excellent molecular markers for age grading in *Aedes aegypti*. The objectives of this study are to examine gene expression profile changes associated with ageing and bloodfeeding in *Anopheles gambiae*, and then to identify molecular markers that may be used for *An. gambiae* mosquitoes age grading. We used Affymetrix Anopheles Genome Array GeneChip® and examined the genome-wide gene expression profile changes associated with ageing through comparisons of gene expression patterns for mosquitoes at days 1, 4, 10, 19, and 28 post emergence. The examination of gene expression changes due to blood feeding involves mosquitoes bloodfed at these age points. We compared the global gene expressions in non-blood-fed adult female mosquitoes at these age points, and found 9,116 transcripts differentially expressed at $P < 0.001$. We then excluded genes which expression was affected by blood feeding, and identified a total of 299 candidate genes. The majority of these 299 genes codes for proteins involved in metabolic process and oxidation reduction. The K-means cluster analysis identified 40 clusters. We chose 6 genes as most promising candidate genes for age grading from cluster 28 because they showed a linear reduction in expressions with mosquito ages. Using the *An. gambiae* mosquitoes from laboratory cages, we generated a calibration model and estimated 95% confidence for mosquito age predictions. The calibration mode, based on the canonical redundancy analysis, produced a R^2 value of 0.79 between the calculated redundancy variates and observed age. We are currently validating the expression profile based age grading method using *An. gambiae* mosquitoes reared in semi-natural MalariaSphere in western Kenya.

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POLYMORPHISMS IN *ANOPHELES GAMBIAE* IMMUNE GENES ARE ASSOCIATED TO MALARIA RESISTANCE

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In nature Anopheles mosquitoes are found to have varying levels of resistance to the malaria parasite, a characteristic known to be under strong genetic control. Understanding the molecular basis of their immunity will give essential insights for novel malaria control strategies. This study aims to uncover single nucleotide polymorphisms (SNPs) associated with mosquito resistance to malaria in the natural vector/parasite couple *Anopheles gambiae*/*Plasmodium falciparum* through genotype to phenotype associations. *An. gambiae* M form mosquitoes from Cameroon were experimentally infected with a sympatric wild *P. falciparum* isolate. The number of oocysts/midgut at day 8 post blood meal was counted giving each mosquito a quantitative phenotype. These mosquitoes were then genotyped for selected SNPs in known immunity genes and statistical tests applied to determine association (genotype/phenotype). 6 out of 157 SNPs show an association to phenotype, located within or upstream of SP Snake-like, TOLL6, SP PPO activate, CLIPB4,

AgMDL1 and CEC1. These 6 SNPs were then tested for association in 2 subsequent infections (same mosquito colony infected with different local wild parasite isolates) where 2 out of the 6 SNPs showed association in the second infection and none in the third. The SNP located within the gene SP Snake-like shows the same trend in all three infections with the homozygous G allele mosquitoes harboring the lowest number of oocysts. This study reinforces the importance of genetic variability in mosquito immunity. The SP Snake-like G allele confers partial resistance and although not significant in all infections, it is likely to play an important role in the mosquito immune response to the parasite. As associated SNPs do not show association to all parasite isolates it suggests their role in immunity is parasite genotype specific to varying degrees. From these results the effect of genotype appears to have a strong effect on immunity and will have to be seriously considered in the development of novel control strategies.

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DUPLICATION AND CONCERTED EVOLUTION OF VITELLOGENIN GENES IN MOSQUITOES

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The mosquito vittellogenin (Vg) genes belong to a small multiple gene family that encodes the major yolk proteins precursors required for egg production. Several Vg genes have been cloned and characterized from several mosquito species, but, their origin and molecular evolution are poorly understood. We isolated four distinct full length Vg genes from the West Nile virus vector *Culex tarsalis* that likely arose from double duplication events. These genes are organized in two pairs (Vg1a, Vg1b; Vg2a, Vg2b). The sequence comparison indicated that Vg1 and Vg2 genes shared 64.3%-65.5% nucleotide identity. Within each pair, the Vg genes shared very high nucleotide identity (98.1% and 97.0%, respectively). For comparative purposes, the Vg genes were identified from the publically-available *Cx. pipiens*, *Aedes aegypti* and *Anopheles gambiae* genome sequences. Vg gene organization in *Cx. pipiens* was very similar to *Cx. tarsalis*, and indicated that the double duplication event was ancestral to the separation of these two species. In contrast, *Ae. aegypti* and *An. gambiae* had three Vg genes that evolved from independent duplication events in each genus. Signatures of concerted evolution were detected in *Culex* and *Anopheles* Vg gene sequences, but not in *Aedes*. In conclusion, these analyses indicate that the evolution of Vg genes is dominated by independent duplication events in 3 different mosquito genera, and that concerted evolution may contribute to sequence homogenization in some genera.

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RESPONSE OF MOSQUITO PROTEIN INTERACTION NETWORK TO THE DENGUE INFECTION

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Two fifths of the world's population is at risk from dengue. The absence of effective drugs and vaccines leaves vector control as the primary intervention tool. Understanding dengue virus (DENV) and host interactions is essential for the development of novel control strategies. The availability of genome sequences for both humans and the mosquito vector greatly facilitates genome-wide studies of DENV-host interactions. We developed the first draft of mosquito protein interaction network using a computational approach. The high-confidence network includes 4,214 *Aedes aegypti* proteins with 10,209 interactions, among which 3,500 proteins are connected into an interconnected scale-free network. We demonstrated the application of this network for the further annotation of mosquito proteins and dissection of pathway crosstalk. More importantly, protein interaction network makes the foundation for systems biology study of DENV-mosquito interactions. Using three datasets based on physical interaction assay, genome-wide RNA interference (RNAi) screens and microarray assay, we identified 705 putative DENV-associated mosquito proteins. The integrated analysis of these proteins and mosquito network revealed three main modules with function in replication/transcription/translation, immunity and transport that were targeted by DENV. Putative DENV-associated proteins were further selected for validation by RNAi-mediated gene silencing, and the results showed the dengue viral titer in mosquito midgut was significantly reduced for 57% of these genes. Our results indicate presence of common host requirements of dengue viruses in mosquitoes and humans. We discuss the significance of our findings to pharmacological intervention and genetic modification of mosquitoes for blocking dengue transmission.

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ETIOLOGY OF FEBRILE ILLNESS AMONG HOSPITALIZED HIV-INFECTED AND HIV-UNINFECTED ADULTS AND ADOLESCENTS IN NORTHERN TANZANIA

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Few studies describe patterns of HIV co-infections in African hospitals in the ART era. We prospectively studied febrile admissions to two hospitals using modern laboratory methods. We enrolled consecutive admitted patients aged ≥13 years with oral temperature ≥38.0ºC during one year in Moshi, Tanzania. A standardized clinical history and physical examination was done and hospital outcome recorded. HIV antibody testing, aerobic and mycobacterial blood cultures, and malaria film were done. HIV-infected patients also received serum cryptococcal antigen testing and CD4-positive T-lymphocyte count (CD4 count). Of 411 patients enrolled, the median (range) age was 37 (14-97) years, 222 (54.0%) were female, and 159 (38.9%) were HIV-infected. Of HIV-infected patients the median (range) CD4 count was 110 (1-1,140) cells/mm^3, 17 (10.7%) had positive serum cryptococcal antigen tests, and 55 (34.6%) were receiving ART and trimethoprim-sulfamethoxazole prophylaxis. Of 374 (90.1%) with blood cultures, 72 (19.3%) grew a pathogen. Of blood cultures with pathogens, 26 (36.1%) grew *Salmonella* Typhi; 10 (13.8%) *Mycobacterium tuberculosis* complex; 10 (13.8%) *Escherichia coli*; 8 (11.1%) *Streptococcus pneumoniae*; 6 (8.3%) *Cryptococcus neoformans*; and 12 (16.7) grew other pathogens. *Plasmodium falciparum* was identified on blood film of 7 (1.7%). HIV infection was associated with *M. tuberculosis* (odds ratio [OR] undefined, p <0.001) and *C. neoformans* (OR undefined, p=0.002) bloodstream infection (BSI), but not with *E. coli, S. pneumoniae,* or *P. falciparum* BSI. HIV infection appeared to be protective against *S.* Typhi BSI (OR 0.12, p=0.001). Forty-three (10.5%) of patients died in hospital. In conclusion, *M. tuberculosis* and *C. neoformans* are leading causes of blood stream infection in Tanzania in the ART era and are closely associated with HIV infection; we demonstrate a protective effect of HIV against *S.* Typhi BSI in this setting. HIV co-infections continue to account for a large proportion of febrile admissions in Tanzania.

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PROPHYLACTIC EFFECT OF TRIMETHOPRIM-SULFAMETHOXAZOLE ON MALARIA IN HIV-INFECTED CHILDREN LIVING IN KAMPALA, UGANDA

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Daily trimethoprim-sulfamethoxazole (TS) reduces malaria risk substantially, but this effect may be compromised as antifolate resistance increases or when adherence is sub-optimal. We assessed the incidence of malaria and the prevalence of resistance-conferring mutations in members of two cohorts: 517 HIV-uninfected and 292 HIV-infected children aged 1-10 years. Daily TS and antiretroviral therapy (ART; based on WHO criteria) was prescribed for HIV-infected participants. HIV-uninfected participants did not receive TS. All participants were provided insecticide-treated bednets. Standardized protocols were used for malaria ascertainment and identification of resistance-mediating polymorphisms. The protective efficacy of TS was compared for three consecutive 9.5-month periods in 2006-08 using negative binomial regression models. Among HIV-infected subjects, 3-day TS adherence data were collected monthly and classified as optimal (no missed doses), inadequate (1-2 missed doses) or very poor (>2 missed doses). Generalized estimating equations were used to assess the relationship between adherence and malaria risk adjusting for repeated measures, CD4 level, age and ART use. TS use had a protective efficacy of 80% (0.10 vs. 0.45 episodes PPY, p<0.001) despite high prevalence of 5 resistance-mediating mutations (dhfr 51I, 108N, C59R; dhps 437G, 540E) in parasites infecting HIV-infected (92%) and uninfected (86%, p=0.22) subjects. The dhfr 164L mutation, which mediates high-level resistance, but is rare in Africa, was seen in 8% of HIV-infected, but only 1% of HIV-uninfected participants (p=0.001). TS efficacy and prevalence of mutations did not change over time. The odds of malaria were higher among HIV-infected subjects with very poor (OR 3.48, p<0.001) or inadequate (OR 2.96, p=0.024) compared optimal adherence. In summary, TS demonstrated good prophylactic efficacy against malaria despite high prevalence of markers of antifolate resistance, efficacy was diminished by poor drug adherence, and TS use was associated with increased prevalence of dhfr 164L.

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THE EFFECT OF UNTREATED HIV INFECTION ON MALARIA: DOWN-REGULATING INNATE INFLAMMATORY RESPONSES

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The majority of malaria cases, like HIV, occur in sub-Saharan Africa, where many individuals are infected with both pathogens. Co-infection with *Plasmodium falciparum* malaria and HIV is a growing concern, as co-infected individuals experience worse clinical outcomes, including accelerated HIV replication (potentially increasing transmission) and the development of higher parasite burdens and complications caused by severe malaria. However, the underlying mechanisms responsible for the adverse clinical outcomes in co-infected individuals have yet to be fully investigated. Severe malaria is characterized by robust pro-inflammatory host immune responses to infection. We hypothesized that HIV co-infection compromises the function of immune cells in response to malaria. Our aim was to examine the phagocytic capacity and inflammatory response of peripheral blood mononuclear cells (PBMCs) from therapy naïve HIV-infected donors to malaria parasites. Freshly isolated PBMCs from therapy naïve HIV- and HIV+ individuals were cultured in the presence of *P. falciparum* over the course of six days. Compared to HIV- individuals, PBMCs from patients with chronic HIV infection showed a marked decrease in the production of TNF (16.84 vs 75.88 pg/ml), IL-6 (149.55 vs 427.75 pg/ml) and IFN-γ (61.08 vs 944.5 pg/ml) in response to malaria parasites. Moreover, monocyte-derived macrophages from HIV+ patients displayed a 27-38% (depending on the parasite strain used) reduction in phagocytic capacity for *P. falciparum* parasitized erythrocytes versus those from HIV- individuals. HIV-1 may therefore impair inflammatory and phagocytic capacity of innate effector cells to *P. falciparum* malaria and contribute to higher parasite burdens and ineffective immune responses in co-infected individuals.

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FETAL IMMUNE PRIMING TO PARASITIC ANTIGENS, IMMUNE ACTIVATION AND SUSCEPTIBILITY TO *IN VITRO* HIV INFECTION

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Mother-to-child-transmission of HIV (MTCT) remains a major problem in many developing countries. While a variety of interventions have contributed to a marked decrease in the rate of MTCT in the U.S. and Europe, in many countries of sub-Saharan Africa the rate remains higher. Though many maternal factors that increase the likelihood of transmitting HIV to offspring have been studied, fetal factors that may increase susceptibility have been less well characterized. We hypothesize that fetal immune activation due to prenatal exposure to parasitic antigens may result in increased MTCT susceptibility. We have previously demonstrated that cord blood mononuclear cells (CBMC) from up to half of newborns of mothers living in a malaria endemic region demonstrate recall responses to malaria antigens, thereby establishing that prenatal exposure and sensitization has occurred. Further, our and other studies indicate HIV positive pregnant women infected with helminths and/or malaria demonstrated an increased risk for MTCT compared to those without parasitic coinfections. We show here that cryopreserved CBMC from Kenyan newborns demonstrate increased susceptibility to *in vitro* HIV infection compared to that of North Americans (21/60 vs 3/25, p<0.04). Viral replication occurs earlier and reaches a greater magnitude in infected Kenyan samples compared to those from North Americans. When Kenyan samples are grouped according to evidence of prenatal malaria exposure, the sensitized group demonstrated increased susceptibility compared to both Kenyan not sensitized and North American groups (14/29 vs 5/29, p<0.025; 14/29 vs 3/25, p<0.01). Interestingly, PCR amplification of viral strong-stop DNA reveals no differences between the groups 24 hours post viral exposure, suggesting that viral replication is enhanced later in the viral life-cycle. These results support the hypothesis that neonates experiencing prenatal exposure and immune response to malaria antigens may be more susceptible to MTCT of HIV.

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SPECIES-SPECIFIC EFFECTS OF DEWORMING AMONG HIV AND HELMINTH CO-INFECTED INDIVIDUALS

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In many resource-limited settings, HIV infected individuals are frequently co-infected with other endemic pathogens, including helminths. A recently published systematic review and meta-analysis of randomized trials suggests that treatment of helminth co-infection significantly attenuates increases in plasma viral load and significantly increases absolute CD4 counts. Given differences in species-specific prevalence rates and the variability of the host response to different helminth species, it is important to determine the relative benefit of eradicating specific helminth species

in HIV co-infected individuals. A search strategy was developed using the methods of the Cochrane Collaboration. Randomized controlled trials and observational studies evaluating helminth eradication in HIV co-infected individuals were identified and additional data were requested from authors when necessary. Data for each helminth species were pooled and analyzed using standardized mean differences. Pooled analyses suggest beneficial effects of deworming on HIV RNA levels for all helminth species (-0.23 $\log_{10}$ copies/mL, p= 0.0004) and for *A. lumbriocoides* (-0.38 $\log_{10}$ copies/mL, p = 0.02). Although no benefit of deworming was shown for schistosomiasis, hookworm, whipworm, strongyloidisis, lymphatic filariasis, or Mansonella, only one randomized trial and one observational study achieved greater than 80% power to detect differences in viral load of 0.3 log copies/mL or differences in CD4 counts of 25 cells/mm^3. In conclusion, analysis of all available randomized and observational study data evaluating the effects of deworming in helminth and HIV co-infected individuals suggests possible benefit in delaying HIV disease progression. While available data suggest greatest benefit with eradication of *A. lumbricoides*, most studies have lacked adequate power to detect clinically meaningful differences in outcomes. Further studies are needed to document species-specific effects following deworming of HIV infected individuals.

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REASONS FOR SWITCHING TO SECONDLINE ANTIRETROVIRAL THERAPY AMONG PATIENTS ATTENDING KABALE HOSPITAL/JCRC KABALE HIV CLINIC

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In 2002, we began the first Anti-Retroviral Therapy (ART) clinic in Kigezi region {population 1 million}. At first clients had to pay for their drugs and laboratory tests. In 2004,in response to the 'three by five' initiative, Uganda introduced new approaches:free drugs,use of clinical criteria to initiate and monitor ART and rolling out to as many health facilities as fast as practicable. This caused a sharp increase in the numbers accessing ART, however, there were inadequate facilities for monitoring. It is only recently that monitoring tests became available to most clients. The objectives of this study were: (1) to document the reasons behind the switching of patients from first line ART to second line ART at the centre, (2) to assess the clinical,immunological and virological performance of patients on the different first line ART regimens; and (3) to compare duration of therapy before switching among the different first line regimens. Of the 1602 clients actively on ART, 70 were on second line medication and of these, 62 qualified for inclusion. The profile of the first line medications: d4T(Stavudine)+3TC(Lamivudine)+NVP(Nevirapine) = 42(67.7%); AZT(Zidovudine)+3TC+EFV(Efavirenz) 10(16.1%); AZT+3TC+NVP 7(11.3%); d4T+3TC+EFV 2(3.2%); and, TDF(Tenofovir)+FTC(Emitricitabine)+NVP 1(1.6%). There were reasons noted to support switching (Rx = treatment, VLG=virological, Imm=immunological, ALG=allergy, LPD=lipodystrophy, PN=peripheral neuropathy): ALG to NVP 22.6%; Rx failure 16.1%; Rx,Imm.&VLG failure 11.3%; VLG failure 9.7%; LPD 9.7%; Rx&VLG failure 8.1%; Imm.&VLG failure 6.5%; Rx&Imm. failure 6.5%; Imm. failure 3.2%; Rx failure&anemia 1.6%; Rx failure,LPD%PN 1.6%. The mean duration of first line therapy before switching: D4T+3TC+EFV=51 months; D4T+3TC+NVP=35 months; AZT+3TC+EFV=26 months; and AZT+3TC+NVP=14 months. In conclusion: (1) treatment failure followed by allergic reactions to ART were the two commonest causes of switching to second line therapy; (2) patients on the D4T+3TC+EFV combination lasted longest before needing a switch; and (3) The 3 by 5 initiative was a very noble strategy that has saved millions of lives through increased access to ART, however, we need to put more resources into laboratory monitoring of patients on treatment as sometimes they seem well clinically yet they have immunological and/or virological failure. Appropriate intervention will minimise development and spread of drug-resistant virus.

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PREVALENCE AND CORRELATES OF HELMINTH INFECTION IN HIV SERO-POSITIVE KENYAN ADULTS

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Deworming HIV and helminth co-infected individuals may slow HIV disease progression. It is important to identify HIV infected individuals who may benefit from antihelminth treatment. We describe the prevalence and risk factors of helminth infection in a cohort of HIV infected Kenyan adults and correlates of helminth clearance after albendazole treatment. HIV infected individuals were screened for helminths at ten sites in Kenya. A cross-sectional analysis of demographic and laboratory data was performed. A subset of helminth infected individuals was re-evaluated twelve weeks after albendazole therapy. Of 1546 HIV sero-positive individuals screened, 286 had evidence of helminth infection. Hookworm species were the most common helminths identified (56.6%), followed by *Ascaris lumbricoides* (17.7%), *Schistosoma mansoni* (9.6%), *Trichuris trichiura* (7.9%), and *Stongyloides stercoralis* (1.4%). Infection with multiple species was infrequent (6.8%). Individuals in urban or peri-urban areas were 60% less likely to be infected with at least one helminth species compared to individuals in rural areas (p=.025). Lack of education, obtaining drinking water from a lake, river or pool and using a latrine were independently associated risks for soil-transmitted helminth infection ($p<0.05$) but not for schistosomiasis. Degree of immune compromise, measured by CD4 count and plasma HIV RNA, was not correlated with helminth co-infection. In conclusion, among HIV sero-positive adults, traditional risk factors, including poor sanitation, lack of access to clean water and poverty remain the strongest predictors of helminth infection. HIV immune status does not correlate with the risk of soil-transmitted helminth infection or shistosomiasis. Empiric anti-helminth therapy is likely to be cost-effective given the high prevalence of helminth and HIV co-infection and accumulating evidence of benefit. However, until the benefit is clear and deworming resource limitations are less, targeted antihelminth therapy addressing species-specific geographic heterogeneity and correlates of co-infection may be an effective strategy.

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PLASMODIUM VIVAX RHOPTRY NECK PROTEIN (*PV*RON2) EXPRESSED AT BOTH ERYTHROCYTIC AND PRE-ERYTHROCYTIC INVASIVE PARASITES

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Host cell invasion is an essential step of the malaria parasites to establish the infection in human and a major target process of the intervention. Recent proteome analysis of the closely-related apicomplexa parasite, *Toxoplasma gondii*, revealed a panel of novel rhoptry neck proteins (RONs), and some of them have been shown to form a complex with Apical Membrane Protein 1 (AMA1) at the "moving junction", an interface of the parasite and the host cell, during invasion. Because most of the RONs and AMA1 are conserved among apicomplexa phylum parasites and *Plasmodium* AMA1 is a leading blood-stage vaccine candidate. Here we characterized *Pf*RON2, one of RONs in *P. falciparum*, the most dangerous

malaria species. *Pf*RON2 transcription peaked at the mature schizont and expressed at the neck portion of the rhoptry in the merozoite shown by immuno-electronmicroscopy. Co-immunoprecipitation of *Pf*RON2 and *Pf*AMA1 from schizont-rich parasites suggested that *Pf*RON2 co-operated with *Pf*AMA1 to invade erythrocyte. Of interest is that, in *P. vivax,* RON2 was detected not only at the rhoptry neck of merozoite, but also in rhoptry of the sporozoite. It should be noted that this is the first time to identify a rhoptry protein in the *Plasmodium* sporozoite. Thus co-operation of RON2 and AMA1 could be extended to the hepatocyte invasion by the *Plasmodium* sporozoite. In summary, RON2 appears to be functional in two essential invasive forms of *Plasmodium* in human, and could serve as a potential target for malaria intervention.

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INDIRECT AMINOACYLATION IN THE *PLASMODIUM* APICOPLAST

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The *Plasmodium falciparum* genome sequence provided many unexpected insights into malaria parasite metabolism and led to the identification of potential drug targets. Using comparative genome analyses, we identified *P. falciparum* genes encoding glutamyl-tRNA synthetase (GluRS) and glutamyl-tRNA amidotransferase (Glu-AdT), enzymes that constitute an indirect aminoacylation pathway for the production of Gln-tRNA^{Gln}, a key substrate required for protein biosynthesis. The indirect aminoacylation pathway is the sole route for the production of Gln-tRNA^{Gln} in archaea, most bacteria, and (possibly) plastids, but is not found in the eukaryotic cytosol. Glu-AdT is an essential enzyme in bacteria and Glu-AdT inhibitors possess anti-bacterial activity but are not toxic to mammalian cells. Bioinformatic analyses suggest that the *Plasmodium* GluRS and Glu-AdT orthologs are targeted to the apicoplast. Molecular genetic and biochemical approaches are being used to characterize the *Plasmodium* enzymes, determine their sub-cellular localization, and evaluate their importance to parasite survival. *P. falciparum* GluRS, the first enzyme in this two-step pathway, was expressed in a wheat germ *in vitro* translation system. Recombinant GluRS glutamylated the cognate substrate apicoplast tRNA^{Glu} and the non-cognate substrate apicoplast tRNA^{Gln}. The latter activity is diagnostic of GluRSs that participate in indirect aminoacylation, confirming that this pathway is functional in *Plasmodium*. Subcellular localization was assessed by transfecting *P. falciparum* parasites with episomal constructs encoding the putative apicoplast-targeting sequences from the A and B subunits of Glu-AdT fused to GFP. In both cases GFP co-localized with acyl carrier protein (ACP), a finding consistent with apicoplast localization of the enzyme. Further studies are underway to characterize the apicoplast indirect aminoacylation pathway and determine whether it is essential for parasite growth. These studies may validate the indirect aminoacylation pathway as a potential target for drug therapy of malaria.

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FUNCTIONAL ANALYSIS OF G-PROTEIN COUPLED RECEPTOR HOMOLOGUES IN *PLASMODIUM*

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The genome of *Plasmodium* encodes a number of large multipass-transmembrane (TM) domain proteins that contain features indicating a role as integral membrane receptors or channels. We have identified two of these as homologues of recently-identified G-Protein Coupled Receptors in the plant, *Arabidopsis thaliana.* They are phylogenetically conserved in a range of mammals, insects and plants and in some, but not all, *Apicomplexa* and other protozoa. To investigate a role in the initiation of signal transduction pathways in *Plasmodium*, we have generated gene knockout lines in a rodent malaria model and performed phenotypic analysis throughout the parasite life-cycle in the mouse and mosquito. Parasites lacking putative *Plasmodium* G-Protein Coupled Receptors are significantly less virulent than wild-type parasites and show alterations in growth and differentiation rates. Assays to identify endogenous ligands and the nature of downstream signaling pathways will be described.

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A NEW ASSAY FOR SILMUTANEOUS DETECTION OF MUTATIONS ASSOCIATED WITH *PLASMODIUM VIVAX* DRUG RESISTANCE

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Renewed interest in *Plasmodium vivax* malaria has been sparked recently by the description of severe cases of the disease, reemergence in different areas, and reports of drug resistance. In Papua New Guinea (PNG), *P. vivax* is the second most prevalent malaria parasite, but as in many endemic countries, national malaria policy focuses on the treatment of *P. falciparum*. As a consequence, *P. vivax* is exposed to artemisinin derivatives, amino-4-quinoleines and sulfadoxine-pyrimethamine using regimens that may be suboptimal for this parasite, thus potentially contributing to the development of drug resistance. To investigate changes in parasite populations following implementation of new treatment regimens, we developed a new assay allowing the simultaneous detection of *P. vivax* single nucleotide polymorphisms (SNPs) associated with drug resistance, in *pvdhfr* (12 alleles), *pvdhps* (4 alleles) and *pvmdr1* genes (2 alleles). This assay is based on a multiplex ligase detection reaction-fluorescent microsphere technology applied on the products of a nested multiplex PCR. It was validated using wild type and mutant sequenced isolates, and samples from PNG field studies were analyzed. The assay detected all 3 genes in 200 of the field samples (85.5%). Three (1.5%) of these samples displayed a mutant allele for *pvdhps*, 167 (83.5%) for *pvdhfr* and 111 (55.5%) for *pvmdr1* genes. Only 23 samples (11.5%) had a wild type allele for all 3 genes. 153 samples (76.5%) displayed a mixed infection and, overall, we observed a mean multiplicity of infection of 2. These preliminary results are consistent with both the rate of mutations previously observed in PNG and the rate of polyclonal infections. This new assay will be used for a country-wide assessment of the prevalence of molecular markers of resistance and to compare parasite populations between regions where different drug pressures are known to have occurred. This data will help to improve our understanding of the relationship between drug pressure and selection of resistant parasites in PNG and other areas endemic for *P. vivax*.

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PRESENCE OF MULTIDRUG RESISTANCE GENE 1 (*PFMDR1)* ALLELES IN *PLASMODIUM FALCIPARUM* SAMPLES FROM A CLINICAL TRIAL TO TEST TWO ANTIMALARIAL DRUGS IN THE PERUVIAN AMAZON

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Malaria falciparum multidrug-resistant is a public health problem in Peru. Mutations (1034S, 1042N, 1246D) of the *pf*mdr1 gene in *Plasmodium falciparum* and the number of copies are used as molecular markers for drug resistance. The wild type *pf*mdr1 (SND) undergoes mutations causing mutant alleles (CDD, SDD and CDY) that increases parasite sensitivity to various antimalarials drugs. Monitoring these mutant alleles will indicate

the possibility of success of an antimalarial. The aim of this report was to identify the proportion of mutant alleles in samples with positive diagnosis of *P. falciparum* before and after treatment with 2 antimalarials in 522 patients treated either with dihydroartemisinin-piperaquine (DHA-PIP) or Mefloquine-Artesunate (MAS). 20 patients were PCR-confirmed positive to *P. falciparum* (15 identified by the clinic and 5 identified by PCR during the follow-up). 90% (18/20) were treated with DHA-PIP and 10% (2/20) with MAS. Direct sequencing of the PCR products was performed in both directions for each PCR product using an ABI-3100 sequencer. Sequencing analysis of 17 samples taken before DHA-PIP treatment indicates the presence of SDD allele in 29.4% (5/17), the CDD allele in 64.7% (11/17) and allele CDY in 5.9% (1/17). The post-treatment analysis indicates the presence of CDD allele in 58.8% (10/17), the wild type SND in 5.9% (1/17), while the SDD and CDY alleles were presented in 29.4% and 5.9% respectively. From 19 samples 1 switched from SDD to SND. The sequencing analysis of 2 samples taken before and after MAS treatment indicates the presence of SDD and CDY allele in each sample. The analysis of 19 samples obtained before treatment presented any *pf*mdr1 mutant allele (SDD, CDD and CDY), all of them common in this region. These alleles possibly detected prior to therapy were removed and the presence of these mutants in a 94.12% of the samples obtained after treatment may be due to a new infection or undetected parasitaemia. The presence of the (mutant or wild) alleles in this area may explain the effectiveness of current therapies.

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SELECTION OF KNOWN RESISTANCE-MEDIATING POLYMORPHISMS BY ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE/SULFADOXINE-PYRIMETHAMINE, BUT NOT BY DIHYDROARTEMISININ-PIPERAQUINE IN BURKINA FASO

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Artemether-lumefantrine (AL), dihydroartemisinin-piperaquine (DP) and, in West Africa, amodiaquine/sulfadoxine-pyrimethamine (AQ/SP) currently offer strong antimalarial treatment efficacy, but there is concern that treatment will select for parasite polymorphisms that decrease drug sensitivity. We evaluated the selection of known polymorphisms in the putative transporters *pfcrt* and *pfmdr1* and the antifolate drug targets *dhfr* and *dhps* in parasites that caused new infections within 42 days of prior therapy in children in Burkina Faso. A study of 580 children in 2006 demonstrated 42-day genotype-uncorrected failures for uncomplicated malaria in 30.9% with AL, 11.6% with AQ/SP, and 7.5% with DP. After prior AL, selection of wild type sequence was seen for *pfcrt* K76 (mixed or mutant 72.7% pre-treatment vs. 52.1% in new infections; p=0.008) and *pfmdr1* N86 (36.0% vs. 18.7%, p=0.025) and Y184 (66.7% vs. 45.8%, p=0.009). After prior AQ/SP, selection of mutant sequence was seen for *pfmdr1* 86Y (36.0% vs. 61.5%, p=0.13) and for *dhfr* 51I (30.8% vs. 61.5%, p=0.05), 59R (28.2% vs. 76.9%, p=0.002), and 108N (30.8% vs. 76.9%, p=0.005). After prior DP, selection was not seen for *pfcrt* 76T (mixed or mutant 72.7% vs. 77.8%, p=0.96) or *pfmdr1* 86Y (36.0% vs. 33.3%, p=0.84) or 184F (66.7% vs. 77.8%, p=0.39). The initial study included only 9 new infections after DP. We evaluated a larger sample from a trial in 2007 that included 378 treatments with DP, with 42 day uncorrected failure in 10.9%. After prior DP, selection was again not seen for *pfcrt* 76T (mixed or mutant 66.7% vs. 59.5%, p=0.43) or *pfmdr1* 86Y (38.7% vs. 40.5%, p=0.85), 184F (67.6% vs. 73.0%, p=0.54) or 1246Y (3.6% vs. 8.1%, p=0.50). Full sequencing of *pfmdr1* from 7 new infections that occured within 35 days of prior therapy with measured complexity of infection of 1 identified known polymorphisms at positions 86 and 184 and a small number of additional polymorphisms. Despite its chemical similarity, piperaquine did not select for the same polymorphisms as chloroquine or AQ, suggesting different mechanisms of resistance.

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GENOME-WIDE ASSOCIATION AND SELECTION SCANS IN THE *PLASMODIUM FALCIPARUM* GENOME

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Genetic diversity in *Plasmodium falciparum* underlies the ability of this human pathogen to escape host immunity and develop drug resistance, allowing it to remain an agent of significant morbidity and mortality. Understanding the nature and extent of this genetic diversity is essential for the development of intervention strategies, as well to monitor the response of the parasite once these interventions are applied. We have developed the first genome-wide genotyping array in malaria, with more than 17 thousand single nucleotide polymorphisms, roughly 1 per kilobase across the genome, and used these markers to study 48 parasites from 3 continents. We have characterized genome-wide genetic diversity and identified several novel candidates for positive natural selection using signals of diversity, population divergence, and long-range haplotypes. We have further created a platform and carried out one of the first genome-wide association studies in malaria, using well characterized phenotypes of drug resistance for chloroquine and pyrimethamine. Even with a limited number of samples, we were able to identify with genome-wide significance two well-known drug resistance loci, the pfcrt and dhfr loci, as well as identified novel candidate resistance loci. We will discuss these findings and the approaches to validate candidate loci identified in genome-wide scans.

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TIMP-1 IN RESPONSE TO EGG ANTIGENS PREDICTS HEPATIC FIBROSIS IN HUMAN *SCHISTOSOMA JAPONICUM* INFECTION

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Schistosomes infect 200M individuals and cause significant hepatic fibrosis in up to 20%. Little is known regarding the mechanisms of schistosome-associated hepatic fibrosis and few biomarkers for risk of fibrosis have been identified. We enrolled 611 *S. japonicum* infected Filipinos aged 7-30 years. Individuals were treated with praziquantel (PZQ) at baseline and abdominal ultrasound to quantify hepatomegaly and hepatic fibrosis was performed at baseline and 12 months post-PZQ. Stool for assessment of *Schistosoma japonicum* infection was collected at baseline and 3, 6, 9 and 12 months post-PZQ. We developed a multiplexed assay (FibroPlex) that quantifies predictors (MIP-1a, IL-13, CTGF, BMP-7, IL-13Ra2, TGF-β), effect modifiers (TIMP-1, MMP-1) and outcomes (PICP, Collagen IV, and ICTP) associated with fibrosis. We measured FibroPlex analytes produced by PBMC stimulated with SEA 4 wks post-PZQ and related these to risk of hepatomegaly and fibrosis 1 yr post-PZQ. After adjusting for age, ht, sex, SES, baseline liver span, baseline *S. japonicum*

intensity, *S. japonicum* intensity 1 yr post-PZQ and water contact, the levels of MIP-1a, CTGF, BMP-7, and TIMP-1 predicted subsequent liver span 1 yr post-PZQ (all $P<0.05$). In similar models, only TIMP-1 in response to SEA predicted increased grade of fibrosis 1 yr post-PZQ ($P = 0.004$). Individuals with detectible TIMP-1 had a 90% greater risk of fibrosis 1 yr post-PZQ compared to individuals with undetectable levels (OR= 1.89, $P = 0.005$). We identified several analytes that predict hepatomegaly 1 yr post-PZQ. In addition, we identified one analyte, TIMP-1, that was a significant predictor of hepatic fibrosis 1 yr post-PZQ. Because TIMP-1 inhibits most matrix metalloproteases (MMPs) which are responsible for collagen degradation, these data suggest that schistosome-associated hepatic fibrosis results in part from inappropriate inhibition of collagen remodeling. These date further suggest that TIMP-1 is a promising biomarker for assessing risk of hepatic fibrosis in schistosome endemic areas.

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BASOPHILS PLAY A POSSIBLE IMMUNO-REGULATORY ROLE DURING *SCHISTOSOMA MANSONI* INFECTION IN MICE

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Basophils, once regarded as minor and likely redundant cells are gradually emerging as the Cinderella of immuno- regulation in recent studies. We investigate whether basophils are involved during *Schistosoma mansoni* infection and what is/are the stimulants. Furthermore, does basophils stimulation result in the characteristic cytokine balance (IL-4 -a central immuno- regulatory cytokine activation). Utilizing single male, female and bisexual *S. mansoni* parasites in BALB/c mice infection we revealed basophils maximal peak at week 7 in all three groups, however, only bisexual infected group producing eggs was markedly high in bone marrow. Spleen basophils from same groups revealed a minimal peak at 4th week for all groups returning to basal level by 5th week and thereafter, except for the bisexual group that sharply increased after 6th week and maintained peaking as bone marrow basophils decreased. Spleen granulocytes correlated with spleen basophils for each group. Specific IgE and IgG1 were also shown to be produced significantly from weeks 6 and 7 increasing thereof. Preliminary work on spleen cytokines shows correlation of IL-4 with increase in number of basophils. Eggs remain the principal stimulator of basophils, and tempting to speculate that basophils may play a critical role in immune regulation. Our on-going studies are aimed at uncovering concrete evidence for it.

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INCREASES IN SCHISTOSOME-SPECIFIC IGE AND CD19+/CD23+ B CELLS IN A COHORT OF KENYAN CHILDREN UNDERGOING REPEATED TREATMENT AND REINFECTION WITH *SCHISTOSOMA MANSONI*

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Age prevalence curves suggest that children in areas endemic for *Schistosoma mansoni* develop partial resistance to reinfection with *S. mansoni* in the early teen years, coinciding with the natural death of the worms they likely acquired in early childhood. Previous research by our group has shown that adults can develop resistance to *S. mansoni* reinfection after repeated cycles of treatment, cure, and reinfection. Increased IgE is the immunologic parameter most consistently associated with this development of resistance. To determine if children undergo similar immunological changes after experiencing multiple rounds of treatment-induced worm deaths, we conducted a 2-year longitudinal study in which 8-10 year old Kenyan children were tested for *S. mansoni* every 4 months and treated with praziquantel (PZQ) when positive. All children were recruited from schools within 3 kilometers of Lake Victoria and were positive for *S. mansoni* and received PZQ treatment at baseline. Children that did not become reinfected with *S. mansoni* after the initial PZQ treatment, and were thus deemed putatively "resistant", had significantly higher levels of schistosome-specific IgE at baseline than did those children who became reinfected 2 or more times during the 2-year follow-up. Furthermore, children that received 3 or more PZQ treatments upon reinfection significantly increased their IgE between baseline and 2-year follow-up, while there were no significant changes in IgE over the course of follow-up in the putatively resistant children who received only baseline treatment. The percentage of CD19+/CD23+ B cells, potentially the producers of IgE, also significantly increased between baseline and follow-up in those children that received 3 or more PZQ treatments. These results suggest that B cell activation and anti-schistosomal IgE are associated with resistance to *S. mansoni* in children, and these immunological parameters can be increased by multiple rounds of infections and PZQ-induced cures.

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FACTORS ASSOCIATED TO TOTAL AND SPECIFIC IGE LEVELS IN RESIDENTS OF AN ENDEMIC AREA FOR SCHISTOSOMIASIS IN MINAS GERAIS, BRAZIL

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This study examines the relationship between demographic factors, water contact, reinfection with *S. mansoni* and serological levels of total IgE and specific IgE against SEA (soluble egg antigen, IgE-SEA) and SWAP (soluble worm adult proteins, IgE-SWAP) antigens. Of 301 residents examined in an endemic area in Brazil 65% (CI95% 58.72-71.28) had *S. mansoni* infection, with a geometric mean egg count of 59.3 (CI95% 54.93-63.61). One year after treatment, prevalence was 13.8% (CI95% 6.01-21.50) and geometric mean egg counts 24.3 (CI95% 17.70-30.96). Before treatment, higher number of eggs in the stool were observed in patients in the age group of 15-29 while higher prevalence was observed in the age group of 30-49. After treatment, the intensity, prevalence and reinfection were higher among individuals in the age group of 6-14 when compared to the other groups. Total IgE levels (mg/mL) were significantly decreased from 7.8 (CI95% 6.6-9.6) to 3.8 (CI95% 3.2-4.3) after treatment. Total IgE levels increased with age and were higher in male individuals. Moreover, the lowest median of total IgE levels were observed among schistosomiasis negative individuals when compared to those from reinfected- and non-reinfected groups. Analysis of IgE-SEA and IgE-SWAP showed that significant differences in antibody production were detected according to sex, age groups and intensity of infection. IgE-SWAP levels after treatment were significantly higher in reinfected- and non-reinfected subjects when compared to those presented by egg-negative individuals. A bivariate analysis showed that water contact activities such as crossing stream, washing clothes and dishes were statistically correlated with total IgE levels after treatment and no statistical correlation with both IgE-SEA and IgE-SWAP levels after treatment. These results suggest that water contact activities might have an important effect on total IgE levels after treatment and that higher total IgE levels after treatment seems to be a predictive factor of reinfection by *S. mansoni*. Although differences in the IgE-SWAP production were observed among reinfected, non-reinfected and egg-negative individuals, the use of IgE-SWAP ELISA in predicting reinfection by *S. mansoni* is still inconclusive. Further studies of these groups are being carried out by our group in the study area.

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SCHISTOSOME SOLUBLE EGG ANTIGENS INDUCE ERYTHROCYTE CELL DEATH

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The major pathological consequences of schistosomiasis are associated with soluble egg antigens (SEA), secreted from egg deposits in liver and other organs. The vigorous immune responses induced by egg antigens result in granuloma formation and other pathophysiologic symptoms such as hepatosplenomegaly and anemia. Schistosomiasis-induced anemia could be multifactorial, but the potential link and molecular mechanisms involved in this process are not clear. A recent clinical study has shown that infected individuals with higher egg load are more anemic and that the level of hemoglobin is reversibly proportional to egg count. In the present study, we evaluated the effect of schistosome SEA on the survival of mouse erythrocytes. Erythrocytes incubated *in vitro* with different concentrations (100-750 ng) of SEA display elevated intracellular Ca^{2+} levels measured by Fluo-3 fluorescence in flow cytometry. In other systems, higher cytosolic Ca^{2+} in turn activates Ca^{2+}-dependent K^+ channels leading to cell shrinkage and phosphatidylserine (PS) exposure that can be measured by forward scatter and annexin V binding respectively. Accordingly, SEA-treated erythrocytes display dose-dependent, higher cell shrinkage and increased PS exposure, a potential signal for apoptosis. Further, SEA-treated erythrocytes show higher fluorescence using the *in situ* apoptosis marker CaspACE™ FITC, indicating the involvement of caspase-mediated cell deformation. Taken together, these results clearly provide several lines of experimental evidence for SEA-induced erythrocyte cell death and provide a new insight into schistosomiasis-induced anemia.

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ENHANCEMENT OF PROTECTIVE IMMUNITY AND IMMUNO-MODULATION OF LIVER GRANULOMA FORMATION WITH THE COMBINATION OF HUMAN AND MICE ANTI-IDIOTYPIC VACCINE MODEL IN *SCHISTOSOMA MANSONI* INFECTED MICE

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Schistosomiasis is a direct consequence of the immunological response to ovideposition in the tissue of the host. Vaccination trials have been studied for achieving the highest rates of protection against infection. The regression in pathology has been attributed to modulation due to immunoregulatory events affecting T cell response to egg antigens. Human anti-idiotypic vaccines (H anti-Ids) in experimental schistosomiasis achieving varying degrees of resistance to challenge *S. mansoni* infection and mice IgG1 subclass monoclonal antibodies (M anti-ids: 9F/5D and 3F/9C) were selected from a panel of MAbs due to its high reactivity and strict selectivity to *Schistosoma* antigens. So, the objective of the present work to investigate the induction of protective immunity to *S. mansoni* infection and the immunomodulatory effect of the combined immunization by the two vaccine models injected prior to infection. To further characterize the mechanisms associated with such vaccine models, on the protective immunity; on liver granuloma formation; on the distribution of T cell subsets and serum cytokines profile responsible for initiation of immunopathology, the following study was done: mice were divided into 4 groups; gp. I: Positive control (mice injected with saline); gp. II: vaccinated with H anti-Id alone; gp. III: vaccinated with M anti-id alone and gp. IV: vaccinated with the combination of the two vaccines. Two weeks after the last immunization, all mice were challenged with 50 *S. mansoni* cercariae. Mice were sacrificed 8 weeks post-infection, liver granuloma size; change in worm burden and ova count was evaluated; T cell subsets and ELISA cytokines profile were estimated and compared to the control mice. Vaccination of C57BL/6 mice with M anti-Id have resulted in ~3% protection, with H anti-Id resulted in ~31% protection and with the combination of the two vaccine resulted in ~42% protection. Also, there was marked reduction in granuloma size and decrease ratio of T cell subsets ($CD4^+/CD8^+$) with low levels of cytokines in mice vaccinated with the combined vaccines. These results suggested that mice anti-Id MAbs combined with human anti-Id could mimic at the T cell level the properties of a protective antigenic epitopes of the targeted-cercariae vaccine and the targeted selectivity of SEA-conjugate immuno-suppressors with marked reduction of the immunopathology that resulted in hepatic granuloma formation and subsequent fibrosis.

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A NOVEL RECOMBINANT *FASCIOLA HEPATICA* PROTEIN BELONGING TO THE METHYL TRANSFERASE PROTEIN-LIKE FAMILY IS AN USEFUL ANTIGEN FOR IMMUNODIAGNOSIS AND A POTENTIAL TARGET FOR IMMUNOPROPHYLAXIS

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Fasciola hepatica, the common bile duct fluke, is a prevalent and economically important disease in the husbandry industry. Although fascioliasis is predominantly a disease of domestic animals such as sheep and cattle, it is now emerging as an important chronic disease in humans with 17 million people infected and about 180 million persons at risk in the highly endemic areas of Bolivia and Perú. A 17KDa protein termed Fh4.26 (GenBank Q5I5Y3) was recently identified by means of successive screenings of a cDNA library previously prepared from *F. hepatica* adult worms using a rabbit anti-*F. hepatica* excretion and secretion (E/S) antigens serum and a serum from rabbit with 4 week of *F. hepatica* infection. By structural homology with other related proteins Fh4.26 was classified as a member of the *F. hepatica* methyltransferase protein family and preserves two DM9 Domains previously reported in *Drosophila melanogaster*. cDNA encoding Fh4.26 was cloned into the plasmid pGEX4T and optimally expressed at 0.2mM IPTG as fusion protein with GST using *E. coli* BL21(DE3) cells. The fusion protein was purified by affinity chromatography and the GST tag removed. The immunodiagnostic potential of rFh4.26 was assessed by the antibody detection Enzyme-Linked ImmunoSorbent Assay and Western blot using a large panel of sera from animals infected with the liver fluke *F. hepatica* or the blood fluke *Schistosoma mansoni*. The assay was highly sensitive and revealed that animals infected with *F. hepatica* develop antibodies against Fh4.26 from 4 to 10wk after infection, which suggests that the antigen is expressed either at early or late stages of infection. Sera from animals with *S. mansoni* were highly reactive as well, which indicate that Fh4.26 is a *Fasciola* / *Schistosoma* cross-reactive antigen. A mouse anti-rFh4.26 serum recognizes the native Fh 4.26 on a Liver Fluke Homogenate by Western blot analysis, this serum also served to perform a confocal microscopy analysis which demonstrated that Fh4.26 is expressed on the tegument and vitellaria cells of the liver fluke. Survival of *F. hepatica* within the infected host requires the parasite to actively maintain its protective tegument. Fh4.26 could be one of the antigens responsible for this maintenance which make it an attractive target for immunoprophylaxis or chemotherapy.

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EVALUATION OF NOVEL DIPSTICK ASSAYS FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS IN MOSQUITOES

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Rift Valley fever virus (RVFV) is a mosquito-borne virus that occurs naturally in various areas in Africa and the Middle East. The ability to detect rapidly RVFV within mosquitoes is key to the early identification of areas at risk of disease outbreaks and will facilitate the effective implementation of prevention and control measures both in countries in which RVFV naturally occurs as well as in areas where the virus may be introduced. Because there are currently no field-deployable rapid assays for detecting RVFV in mosquitoes, we developed a hand-held immunochromatographic assay similar to the West Nile and St. Louis encephalitis VecTEST™ assays. We evaluated several combinations of capture and detector monoclonal antibodies, and resultant dipsticks were readily able to detect a single RVFV-infected mosquito in a pool containing 24 virus-negative specimens. The dipstick assay was able to detect 90% of pools containing a single mosquito with a titer $\geq 10^5$ plaque-forming units (PFU) of RVFV (titers typical of a mosquito with a disseminated infection), but failed to detect viral antigen in mosquitoes containing $<10^{4.5}$ PFU (titers found in infected, but non-transmitting mosquitoes). These specimens were also tested by RT-PCR and the dipstick detected RVFV antigen in >90% of specimens with CT values <23, but rarely detected antigen in specimens with CT values >25. No false positives were detected. Because one of the concerns with working with field-collected mosquitoes is the potential for contact with infectious material, we evaluated the effect of the "grinding" medium on infectious virus, and infectious virus was not detected from specimens triturated in grinding medium. This new dipstick assay for detecting RVFV should allow for the rapid detection of RVFV from field-collected specimens without the need for expensive equipment or highly trained personnel.

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USING LUMINEX TO IDENTIFY *CULEX* BLOODMEALS AND EVALUATE TRAP BIAS

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Because vertebrate host species vary in their competence for West Nile Virus (WNV) and mosquito infection with the virus is dependent on high host viremias, bloodmeal identification may play an important role in understanding varying levels of WNV transmission. In recent years, DNA sequencing of mitochondrial genes has been the most common method of bloodmeal identification in mosquitoes. Although sequencing is extremely robust, it can be expensive and time consuming for large host identification projects. With over 6,000 bloodfed mosquitoes collected throughout California and preserved for bloodmeal analysis, species-specific Luminex® probes were developed to quickly and inexpensively identify bloodmeals from the most common avian and mammalian hosts of *Cx. tarsalis* and the *Cx. pipiens* complex, the major vectors of WNV in California. Using a combination of Luminex® and DNA sequencing, preliminary results from one study site, a heronry, suggest that *Cx. tarsalis* feeds predominantly on the most abundant hosts at this site, namely herons and egrets. When these birds fledge and leave the site, bloodfeeding shifts to other avian as well as mammalian hosts. The Luminex®-sequencing combination was also used to evaluate the importance of trap bias in bloodmeal identification studies. Bloodmeals were identified from *Cx. tarsalis* collected by CO_2 traps, gravid traps and from resting boxes at the same study site over the same period of time. In the future, this identification approach will be used to efficiently compare bloodmeal selection in various biomes and environments throughout California.

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EPIDEMIOLOGY OF WEST NILE VIRUS IN SOUTHERN CALIFORNIA: THE ROLE OF *CULEX QUINQUEFASCIATUS* AND HOUSE FINCHES

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Since the invasion of California by West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in 2003, evidence of infection has been continuously obtained in mosquitoes, wild birds, and mammals including humans. We investigated blood feeding behavior of *Culex quinquefasciatus* Say, *Cx. tarsalis* Coquillett, *Cx. erythrothorax* Dyar, and *Cx. stigmatosoma* Dyar in order to evaluate their respective roles in transmission of WNV by using a PCR method based on cytochrome b gene of mitochondrial DNA. Our analyses of engorged mosquitoes collected from three southern counties in California revealed that all aforementioned species acquired blood meals from birds and mammals, emphasizing the important roles for these mosquitoes in both enzootic and epizootic/epidemic transmission of WNV. *Culex quinquefasciatus* acquired greater than one third of blood meals from the house finches (*Carpodacus mexicanus*), suggesting the importance of this WNV-competent avian species in enzootic cycling of the virus in the region. Thus, based on abundance, opportunistic blood feeding on a variety of competent birds and mammals, including humans, vector competence and high infection rate, we conclude that *Cx. quinquefasciatus* is the principal vector of WNV and that house finches play a significant role in virus amplification in southern California. Other mosquito species, such as *Cx. tarsalis* and *Cx. stimatosoma,* are also spatial and temporal contributors to WNV transmission.

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COMMUNAL AVIAN ROOSTS AS AMPLIFICATION FOCI FOR WEST NILE VIRUS IN URBAN AREAS IN NORTHEASTERN USA

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The eco-epidemiological factors leading to spatial heterogeneity in enzootic and epidemic transmission of WNV to humans are not well understood. *Culex pipiens* and *Cx. restuans* have been recognized as the primary enzootic mosquito vectors and American Robins (*Turdus migratorius*) as the most common host early in the transmission season in the northeastern United States. A comparatively lower number of Robin-derived blood meals in mosquitoes later in the transmission season concurrent with the occurrence of most human cases have been attributed in part to a seasonal shift in mosquito feeding to humans and other birds. We propose that the comparatively lower number of Robin-derived bloodmeals later in the season is most likely due to post-breeding use of communal roosting locations in these birds that have been overlooked in prior surveys. By radio-tracking Robins, we located seven Robin and mixed species communal roosts within New Haven, CT an urban focus of WNV. We collected host-seeking and resting mosquitoes around the seven roost locations as well as in three sites located away from roosts. We found that minimum infection rates for WNV in *Cx. pipiens* and *Cx. restuans* were

significantly higher in communal roosts than in non-roost sites during the same time period. Within the roosts, we identified *Cx. pipiens* and *Cx. restuans* feeding on Robins in late summer and fall. By analysis of 115 engorged *Aedes vexans* collected around the roosts we identified avian- (5.2%), and mammalian-derived bloodmeals (94.8%) of which 6.4% (n = 7) were from human hosts. Our collective evidence points to these roosting locations as important late-season amplification foci that facilitate spillover transmission to humans due to high infection rates in the enzootic vectors, as well as greater potential for bridging WNV to humans by opportunistic mosquitoes such as *Aedes vexans*. We propose that these communal roosts may be an overlooked feature in other WNV endemic areas that could explain spatial patterns of WNV transmission.

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WEST NILE VIRUS AFFECTS THE RATE OF BLOOD DIGESTION IN *CULEX PIPIENS QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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Culex pipiens quinquefasciatus were fed blood meals containing a high virus dose (6.2 logs plaque-forming units (pfu) West Nile virus (WNV)/ mL), low virus dose (5.3 logs pfu WNV/mL), or no virus and incubated at 28°C. Twenty mosquitoes per group were collected daily from one to six days post-infection (dpi) and the rate of blood digestion was scored using the Sella scale. Bodies and legs of mosquitoes fed blood meals containing WNV were separated and tested for virus to determine rates of midgut infection and viral dissemination out of the midgut. There were no significant differences in rates of infection, dissemination, or digestion between mosquitoes given blood meals containing a low or high virus dose ($p \geq 0.05$). However, at two dpi, mosquitoes given either virus dose showed significantly faster digestion rates compared to mosquitoes given an uninfected blood meal (χ^2=10.85, df=1, p=0.004). This finding suggests that WNV increases the rate of blood digestion in *Cx. p. quinquefasciatus*. Increased digestion rates in virus-infected mosquitoes may shorten the gonotrophic cycle and increase the chance that an infectious mosquito will take a subsequent blood meal. This phenomenon may serve to facilitate and increase the transmission rate of WNV in nature.

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RELATIONSHIPS BETWEEN MEASURES OF VECTOR COMPETENCE FOR *CULEX PIPIENS QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) INFECTED WITH WEST NILE VIRUS

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Virus growth within mosquito tissues affects vector capacity for virus transmission and can affect our assessment of vector competence if measured at only one time point. To address this, *Culex pipiens quinquefasciatus* were fed blood meals containing 6.9 logs plaque-forming units (pfu) of West Nile virus (WNV) / mL and held at 28°C for extrinsic incubation periods (EIP) of 7 d, 14 d, or 21 d. These EIPs were chosen to represent points in the virus transmission period that were early (i.e. early dissemination out of the midgut), intermediate, and late. We investigated vector competence measured as rates of infection (% with WNV-positive abdomens), dissemination (% with WNV-positive legs or thoraces), and transmission (% with WNV-positive saliva), as well as WNV titer in abdomens, legs, thoraces, and saliva. As has often been observed, preliminary results indicate that rates (percent at 7 d, 14 d, 21 d, respectively) of infection (abdomens = 100, 100, 100%), dissemination (legs = 91, 98, 97%; thoraces = 88, 98, 100%), and transmission (saliva = 2, 27, 25%) increased or were equivalent with increasing EIP. We also observed that titers (logs pfu WNV / mL ± SE at 7 d, 14 d, 21 d, respectively) of abdomens (6.2 ± 0.05, 6.8 ± 0.08, 6.8 ± 0.05) and thoraces (4.9 ± 0.1, 7.0 ± 0.2, 7.1 ± 0.04) were higher than titers in legs (2.9 ± 0.2, 4.8 ± 0.1, 5.0 ± 0.07) and saliva (0.8, 2.7 ± 0.2, 2.2 ± 0.4) during each EIP. Preliminary results suggest that the proportion of mosquitoes with virus in saliva is not correlated with the proportion of mosquitoes with virus in abdomens, legs, and thoraces nor is saliva titer correlated with titer in other tissues. This indicates that these proxy measures are not good indicators of vector competence under the conditions of our test. The causes of limits to virus replication in different mosquito tissues are unknown. However, an understanding of the relationships between various measures of vector competence will improve our ability to characterize mosquito populations for vector competence to ultimately improve risk assessment for disease and develop novel control strategies.

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EVALUATION OF KENYAN MOSQUITO SPECIES AS VECTORS OF WEST NILE VIRUS

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West Nile virus (WNV) is a member of the family Flaviridae (genus *Flavivirus*). It is an arbovirus that was first isolated from the blood of a woman in the West Nile District of Uganda in 1937. Human infection with WNV may cause West Nile fever, a mild and self limiting illness, severe encephalitis or meningitis. Mosquitoes of the *Culex* species are the principal vectors involved in transmission of the virus in the USA. Although the first field isolation of WNV from male *Culex univitattus* mosquitoes was made in Kenya in 1998, thus demonstrating vertical transmission of this virus in nature, little work has been reported on the ability of Kenyan mosquitoes to transmit this virus. We evaluated the potential of several field-collected mosquito species from Baringo and Naivasha: (*Cx.. univittatus, Cx. quinquefasciatus, Cx. vansomereni, Mansonia uniformis* and *Ma. africana*) for WNV. Infection, dissemination and transmission rates were determined by assaying mosquitoes orally exposed to WNV infected chickens. Engorged mosquitoes were incubated for 7-21 days and then allowed to feed on 1-3 day old naïve chicken. The mosquito bodies and legs were assayed separately by plaque assay to detrmine infection and dissemination rates. Chickens were bled 1 day later and plaque assays performed to determine if the mosquito transmitted WNV. All mosquito species tested in this study were susceptible to infection with WNV and developed disseminated infections. *Cx. quinquefasciatus* had the highest infection rate (72%), followed by *Cx. univittatus* (53%) *Cx. vansomereni* (50%), *Ma. africana* (50%), and *Ma. uniformis* (38%). The differences in infection rates between these species were not statistically significant, *p >0.05. Cx. quinquefasciatus* also had the highest transmission rate (44%), followed by *Cx. univittatus* (25%) and *Cx. vansomereni* (17%).The two *Mansonia* species mosquitoes did not transmit the virus. The vector competence of diverse mosquitoes in Kenya for WNV transmission may vary from species to species and more field collected specimen should be tested to identify important vectors.

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CLINICAL AND PRECLINICAL EVALUATION OF DENVAX, A TETRAVALENT DEN-2 PDK-53-BASED CHIMERIC DENGUE VACCINE

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Among the mosquito-borne viruses, dengue viruses (DENVs) are notable for their global distribution and the frequency of large scale epidemics that they cause. To date, there is no effective vaccine to prevent against DENV infections. DENVax is a tetravalent DENV vaccine based on using the DENV-2 vaccine, strain PDK-53, as a vector. Three chimeric recombinant viruses were constructed, each bearing the vector`s capsid and non-structural gene backbone while expressing DENV-1, DENV-3 or DENV-4 prM (pre-membrane) and E (envelope) structural genes. The safety, immunogenicity, and protective efficacy of GMP-quality DENVax formulations were tested in mice and in monkeys. In these studies, we identified tetravalent formulations that; i) were safe and caused limited viremia after vaccination, ii) induced neutralizing antibodies to all four DENV serotypes, and iii) conferred protection in both species. Toxicology studies were performed in AG129 mice that lack the interferon-αβ and interferon-γ receptors and are permissive for dengue virus replication. DENVax was safe in these mice, generating only mild, transient physiological changes relative to wild type (wt) DENV-2. Similarly in monkeys there were no notable changes in body weight, temperature and hematological parameters following vaccine administration. Levels of viremia after initial vaccine inoculation were low and limited in duration. Viremia was exclusively (monkeys) or predominantly (AG129 mice) limited to the DENV-2 PDK-53 component; the chimeras were significantly less viremic. Despite the low viremia, neutralizing antibodies to all four DENV serotypes were induced in both mice and monkeys. Immunized mice were 100% protected against lethal challenge with wt DENV-1 or DENV-2. Immunized monkeys were also protected from DENV-induced viremia after wt DENV-1, -2, -3, or -4 challenge. These preclinical studies set the stage for human clinical testing of the safety of DENVax formulations in dengue naïve adult volunteers. Clinical testing of the tetravalent DENVax vaccine is being initiated in the U.S. and in the developing world; protocols and progress of the trials will be reviewed.

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EVALUATION OF NEUTRALIZING ANTIBODY RESPONSES AGAINST A LARGE RANGE OF WILD-TYPE ISOLATES IN SERA OF PRIMATES VACCINATED WITH A TETRAVALENT DENGUE VACCINE

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A live-attenuated, tetravalent dengue vaccine (TDV) based on chimeric viruses made by deleting prM/E genes of YF17D vaccine and replacing by the DENV prM/E genes is in phase 2 clinical trials. Serum-neutralization is routinely evaluated against DENV wild-type (wt) strains from which TDV E genes were derived (DENV-1/PUO-359, DENV-2/PR-159, DENV-3/PaH881, DENV-4/TVP-980). The potential of vaccine-elicited antibodies to neutralize a spectrum of circulating DENV strains was first investigated by aligning the DENV E gene sequence of each of the four TDV serotype viruses with sequences retrieved from public databases according to their year and place of isolation. Intra-serotype variation in this genomic region is very low and no significant differences were seen. In addition, each parent vaccine sequence had ≥99% identity with consensus sequence of its related serotype. The few unmatched residues were introduced into a three-dimensional TDV E model and most of them showed no potential impact on antigenicity based on domain location, accessibility and biochemical changes. We concluded from this *in silico* study that the anti-E antibodies elicited against the vaccine viruses have the potential to neutralize a wide range of DENV strains. A second approach was conducted to confirm experimentally these observations. Recent DENV-1-4 field isolates (≥ 10 strains per serotype) were collected from various endemic areas (Asia-Pacific, Africa, Caribbean, South America, and Martinique) and sequenced to determine their genotype. All the viruses were isolated on mosquito cells and were used at low-passages (two to three amplifications on mammalian cells). A pool of sera obtained from monkeys immunized with two or three doses of TDV was tested in parallel against homologous (parent DENV) or heterologous (wt isolates) strains for its DENV neutralizing ability. In an assay including 16 Asian strains isolated in the last 5 years, similar neutralizing titers were seen against all strains, supporting the hypothesis that TDV-elicited antibodies have the potential to cross-neutralize recent wt DENV strains. Implications for a human vaccine testing strategy will be discussed.

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SAFETY AND IMMUNOGENICITY OF A TETRAVALENT DENGUE VACCINE IN FLAVIVIRUS-NAIVE AND -IMMUNE PEDIATRIC POPULATIONS WITH TWO VACCINATION REGIMENS

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A tetravalent dengue vaccine for use in endemic areas should be well tolerated and immunogenic in children previously exposed to flaviviruses (FV) and FV-naïve subjects. Seventy-two Filipino children aged 2-11 years old were randomized to two groups: Group 1 (n=48) received three tetravalent dengue vaccine (TDV) doses at 0, 3-4 and 12 months. Group 2 (n=24) received two TDV doses at 0 and 8-9 months. Baseline flavivirus serostatus (FV+/-) was determined by the presence of dengue and/or Japanese encephalitis virus antibodies in samples taken at enrollment. Vaccine safety and immune response were evaluated after each vaccination in FV- versus FV+ populations. Immune response was measured by $PRNT_{50}$ for each dengue serotype and expressed as both geometric mean titer (GMT) and percent of seropositive subjects (titer ≥10 [1/dil]). The percent of subjects seropositive for at least three serotypes was also analyzed. Most injection site and systemic reactions were mild or moderate, lasting less >3 days. The reactogenicity profile was similar in FV+ and FV- children, with slightly more frequent mild to moderate fever in FV- population. Adverse events were less frequent after second and third TDV doses. The reactogenicity profile was unaffected by the period between first and second TDV doses. A balanced immune response was obtained against all four serotypes after three doses in FV- children. GMT and seropositivity rates increased after each vaccination in FV+ children. Interestingly, two doses at 0 and 8-9 months induced immune responses similar to three doses at 0, 3-4 and 12. Full statistical analysis is ongoing and specific GMT and seropositivity rates will be presented. In conclusion, TDV was well tolerated and immunogenic in children regardless of previous FV exposure. These data support a longer interval between first and second dose. However, three doses are required to reach a balanced immune response against all four serotypes. With these considerations in mind, a 0-6-12 month three dose schedule months will be used in TDV evaluation trials in endemic areas.

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CLINICAL EVALUATION OF LIVE ATTENUATED DEN3 VACCINE CANDIDATES

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Several molecular genetic strategies have been used to develop a dengue virus type 3 (DEN3) live attenuated vaccine candidate suitable for inclusion in a tetravalent dengue vaccine. Antigenic chimeric viruses have been generated in which the prM-E genes of vaccine candidate DEN4Δ30 have been replaced with the corresponding genes from a DEN3 wild-type virus. Additional DEN3 vaccine candidates have been generated using full-length DEN3 in which the 3'-UTR contains two deletion mutations (Δ30/31) or in which the entire 3'-UTR has been replaced with that derived from DEN4Δ30 (3'D4Δ30). Chimeric vaccine candidate DEN3/4Δ30 (structural genes from DEN3 Sleman/78 isolated from a mild outbreak) has recently been evaluated in volunteers at a single dose of 10^3 pfu (n = 20) or 10^5 pfu (n = 20). Although this vaccine candidate was well tolerated by all volunteers, the level of infectivity was too low (HID_{50} > 10^5 pfu, seroconversion rate 25 - 30%) to justify further development. To test the hypothesis that the prM and E genes from DEN3 Sleman/78 may be responsible for the observed low infectivity of the chimeric virus for humans, an additional chimeric virus was constructed using structural genes derived from a virus isolated from a severe outbreak (DEN3 Sri Lanka/91). In rhesus monkeys, the infectivity of the Sri Lanka/91 and Sleman/78 chimeric viruses was similar, and the Sri Lanka/91 chimeric virus was not considered for evaluation in humans. Initial clinical evaluation of rDEN3-3'D4Δ30 is nearing completion, and the virus is safe and well tolerated by all volunteers. The frequency of viremia (20%), the mean peak titer of virus in blood (0.6 $\log_{10}$ pfu/mL), and the frequency of rash (25%) and neutropenia (10%) are similar to values observed for our highly infectious DEN1, DEN2, and DEN4 vaccine candidates. Neutralizing antibody titers and seroconversion rates are pending and will be presented. An IND application for the clinical evaluation of DEN3Δ30/31 has been submitted.

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SAFETY AND IMMUNOGENICITY OF A 2-DOSE REGIMEN OF RDEN1Δ30 DENGUE SEROTYPE 1 VACCINE WITH BOOSTING AT FOUR VERSUS SIX MONTHS

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Studies of live attenuated tetravalent (LATV) dengue virus vaccine to date have indicated that multiple doses of the vaccine may be required to induce adequate neutralizing antibody responses to all 4 serotypes. In preparation for initiating clinical trials with our LATV, we evaluated the effect of a second dose of the DEN1 candidate vaccine rDEN130 given 4 or 6 months after the first dose. Sixty volunteers were enrolled and randomized to receive a second dose of vaccine or placebo at 4 or 6 months. Twenty-five volunteers in each group received vaccine (1,000 PFU dose subcutaneously) and 5 each received placebo (vaccine diluent). The vaccine was well tolerated. No vaccine-related SAEs were reported. Following first vaccination (n=50), the most common vaccine-related adverse events were a non-pruritic rash (27%) and transient neutropenia (43%), the incidence and severity of which were not significantly different from those observed in a previous trial of rDEN130. No vaccinee developed fever. 67% of vaccinees had detectable viremia (mean peak titer = 1.06 ± 0.1 $\log_{10}$ PFU) following first vaccination and 94% of all vaccinees were infected. The geometric mean titer (GMT) at study day 42 following first vaccination was 99 (reciprocal titer, range <5 - 844). The GMT prior to dose 2 was 39 in the 4-month cohort (day 120) and 36 in the 6-month cohort (day 180). Following the second dose of vaccine at 4 or 6 months (n=46), no vaccinee developed detectable viremia at any time point, nor did any vaccinee develop a flavivirus vaccine-like rash. 4/46 vaccinees (8.7%) became neutropenic following dose 2. None of the vaccinees developed a four-fold rise in DEN1 neutralizing antibody titer following dose 2. GMTs at day 42 post second vaccination were 36 (4-month cohort) and 31 (6-month cohort). In summary, this monotypic DEN1 vaccine vaccine is well tolerated and highly immunogenic, inducing sterilizing immunity to vaccine virus for at least 6 months.

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POTENTIAL IMPACT OF VACCINATION ON THE TRANSMISSION DYNAMICS OF DENGUE: A FOUR SEROTYPE MODEL

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Several models of dengue transmission have been proposed but none combines the 4 following factors that are potentially key in the dynamics of this disease: host-vector interaction, interaction between the 4 serotypes, seasonality and age-structure of the population. This study was undertaken to develop a model of dengue transmission, to explore its ability to reproduce the observed disease dynamics and to assess with a well validated model the potential impact of vaccination. A set of ordinary differential equations is used to represent dengue transmission dynamics in humans and vectors. The model extending Bartley et al. (2002) enables to differentiate primary from subsequent cases and to consider both cross-protection and cross-interference either in isolation or sequentially. Cross-interference refers here to the possibility of an increased susceptibility after a first dengue infection and, in case of secondary infection, to an increased infectiousness or increased risk of severe outcome. Seasonality is generated by varying the vector birth rate. The model was used to reproduce Dengue transmission dynamics using parameters appropriate for Vietnam and Mexico and to assess the potential impact of routine and catch-up vaccination in those two countries. When seasonality and transient cross-protection between serotypes is included, dengue incidence exhibits multiyear cycles that represents well the observed dynamics of dengue. In the case of Vietnam, a 80% effective vaccine administered to 90% of toddlers could potentially reduce dengue incidence by more than 90% in the mid-long run. Both direct and indirect effects of vaccination contribute to this reduction, with a role of similar importance. Catch-up programs targeting children are likely to significantly accelerate the drop in disease incidence: in the most favorable scenario a 90% reduction can be obtained the first year. In conclusion, serotype interactions and seasonality play a key role in generating interannual cycles that are characteristics of dengue transmission. The model also shows the potentially dramatic impact of vaccination on disease control.

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A SYSTEMATIC LITERATURE REVIEW AND EXPERT PANEL'S ASSESSMENT HEALTH ECONOMICS OF DENGUE

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Multiple dengue vaccines are approaching phase 3 clinical trials. Policy makers need cost-effectiveness studies to assess their usefulness. The objective of this study was tetermine gaps in the literature and provide expert opinion for future economic studies. We searched PubMed, EconLit EMBASE, WHOLIS, LILAC and the reference lists of indexed manuscripts to identify dengue-specific health economic research through 2008. We convened a meeting of health economists, representatives from the pharmaceutical industry, and WHO. We identified 39 manuscripts or reports focusing the health economics of dengue. Seven manuscripts reported dengue burden in natural units and all used DALYs. Twelve studies were identified that reported national or regional cost of illness of dengue with most focusing on outbreaks. Five of these 12 examined only the government costs of prevention and control. Four studies reported dengue COI from the perspective of the healthcare system and 6 from the household perspective. There were 2 published studies of potential cost-effectiveness of dengue vaccination compared to either vector control or case management, and 6 additional studies of the cost-effectiveness of vector control. Finally, one reported willingness of the general public to pay for a dengue vaccine, and one reported policymakers' views regarding dengue vaccine implementation. In conclusion,compared to some diseases, there are limited data on the economic burden of dengue. Additional research is indicated. The panel recommended: [1] inclusion of information on adults and private sector patients and representative data from several types of health care facilities in a given country; [2] development of simple first generation economic models of dengue vaccination with clearly stated limitations; [3] development of dynamic transmissions models to assess herd protection from vaccination and the impact of different dengue immunization strategies; and [4] adherence of future vaccine cost-effectiveness studies to new WHO guides for evaluation of immunization programs.

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EHRLICHIOSIS: A CASE REPORT FROM LYNCHBURG VIRGINIA

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Ehrlichiosis, a tick borne disease caused by an obligate intracellular Gram negative bacterium (Anaplasmataceae) is reported from Malaysia, Borneo, Philippines, Japan, Russia, Tibet, and Thailand. Ehrlichiosis parallels the distribution of the tick vectors that transmit *Ehrlichia* spp to the mammalian host. In the USA, distribution of human Ehrlichiosis mirrors that of the definitive host (white footed mouse, white tail deer) and distribution of the tick vectors *Amblyomma americanum* (the Lone Star tick), *Dermacentor variabilis* (American dog or wood tick), and *Ixodes persulcatus.* As obligate intracellular parasites of mononuclear cells and granulocytes, *Ehrlichia* produces cytoplasmic inclusion bodies (morulas) after 5-7 days. As a CDC notifiable disease, ehrlichiosis refers to infections cause by *Anaplasma* or *Ehrlichia* spp. Most cases of ehrlichiosis are asymptomatic, producing mild to moderate acute febrile illness. However, in the immunocompromised human host, ehrlichiosis may be fatal. Serological methods for diagnosis of ehrlichiosis are often inconclusive; therefore, culture of the organism is required to obtain for definitive diagnosis. Case Report: The patient is a 57 year old caucasian in good health, non-smoker, and non-drinker with an active outdoor lifestyle. He is scoutmaster for a Lynchburg, VA scout troop and hikes and camps on a regular basis. The patient first noticed symptoms three weeks prior to admissions to the emergency room. He left work on Tuesday with a severe headache and went to his internist on Friday. He remembered removing a dog tick eight days prior. He was prescribed azithromycin. On Saturday the patient felt ill and lost appetite and decided to discontinue the antibiotic. The patient waited a week for the illness to clear, spent most of the time sleeping, missing work and had to force himself to eat and drink. The following Wednesday he returned to his internist, blood was drawn and sent to the Mayo Clinic for serological testing. On Saturday he was admitted to the hospital, and Lyme Disease and Rocky Mountain Spotted Fever were ruled out. No rash was reported. Temperature fluctuated but never above 99°F. The patient had edema in the extremities and gained six pounds despite not eating. The patient was started on IV fluids and IV Tetracycline to treat what was believed and later confirmed to be ehrlichiosis. He was subsequently given oral tetracycline and released from the hospital.

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KNOWLEDGE AND PRACTICES AMONG YELLOW FEVER VACCINE PROVIDERS AND CLINICS, PENNSYLVANIA, USA, 2008

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In the United States (US), the Centers for Disease Control (CDC) delegates responsibility to state health departments to issue the uniform stamps used to validate the International Certificates of Vaccination required by the International Health Regulations. No evaluation of the Yellow Fever (YF) vaccination program to assess quality and safety issues has been performed in the US. This survey was conducted to determine the level of knowledge and quality of practices of YF vaccine providers and clinics in Pennsylvania (PA). YF stamp owners in PA were those listed on the CDC YF registry. YF providers were considered to be all YF stamp owners and associates they identified. YF providers were invited to complete a web-based or a mail-out paper survey. Site visits were conducted at a geographically stratified sample of YF clinics with probability proportionate to size for each state region. A total of 193 (56%) of 343 YF providers responded to the survey. Among respondents, 67% were physicians and 27% were nurses. On average, 70% (range, 40% to 91%) of providers correctly answered each of the 14 knowledge questions. Ninety percent knew that YF vaccine is a live virus vaccine, while 88% knew that the revaccination interval is 10 years. Fifty percent of providers incorrectly answered that CDC YF vaccine recommendations match destination country vaccine entry requirements. Providers with >5 years of YF vaccine experience were more likely to correctly identify regions endemic for YF than those providers with ≤5 years experience (odds ratio=3.5, 95% CI 1.6-7.6). Site visits were conducted at 21(13%) of 165 PA YF clinics. Of the these clinics, 91% give YF Vaccine Information Statements to all patients at the time of vaccination, but only 57% keep a log of all YF vaccine doses administered. In conclusion, the overall knowledge and practices of YF providers and clinics in PA are high. However, knowledge gaps and practice deficiencies exist, demonstrating the need for development and implementation of a YF vaccine-specific provider training module.

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14-3-3β PROTEIN LEVEL IN THE CEREBROSPINAL FLUID AND SERUM AS AN INDICATOR OF DISEASE ACTIVITIES IN PATIENTS WITH PARASITE-INDUCED EOSINOPHILIC MENINGITIS

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The 14-3-3β protein is a cerebrospinal fluid (CSF) marker of neuronal damage during the development of Creutzfeldt-Jakob disease. In addition, increased 14-3-3β protein is also found in CSF from patients with a variety of neurological disorders. The goal of this study is to determine whether the levels of serum/CSF14-3-3β protein in patients with eosinophilic meningitis could correlate with CSF abnormalities and clinical course. In a cohort study among nine Thai laborers with eosinophilic meningitis due to eating raw snails (*Ampullarium canaliculatus*), we examined the CSF weekly while patients were still hospitalized and followed up the serum for 6 months. The levels of 14-3-3β protein in CSF were analyzed by western blot and an in-house 14-3-3β enzyme-linked immunosorbent assay (ELISA) measurement was established. The correlation between 14-3-3β protein in CSF, laboratory abnormalities, and clinical severity and MRI findings were determined. All of the nine patients received a total of 23 lumbar punctures. The elevated 14-3-3β level was detected in the CSF from eight out of nine (81%) patients during initial hospital admission. After 2 weeks of treatment, all patients showed a declined level or cleared of 14-3-3β protein in the CSF. By developing an in-house ELISA for measurement of 14-3-3β protein, it was found that the serum 14-3-3β level was significantly increased in patients during initial visit. After treatment, the serum 14-3-3β level in meningitis patients was rapidly returned to normal threshold (Fig 1). There was a trend of correlation between initial CSF 14-3-3β level with pleocytosis or eosinophilia in the CSF of patients with eosinophilic meningitis (Spearman's correlation test, r = 0.6, *P = 0.089)*. In conclusion, the serum 14-3-3β concentrations may constitute a useful marker for disease severity and follow up in patients with eosinophilic meningitis caused by *Angiostrongylus cantonensis*.

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MICROBIOLOGICAL ASPECTS OF EROSIVE-ULCEROUS LESIONS IN UPPER PART OF THE DIGESTIVE TRACT IN PATIENTS WITH LIVER CIRRHOSIS AND PORTAL HYPERTENSION

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Microbiological study conducted in patients before and after treatment. Gastric juice, biopsy materials from mucosa of the esophagus and cardia collected. Gastric juices obtained in the morning, on an empty stomach by fractional probe, biopsy materials obtained by esophagogastrofibroscopy. We identified micro flora of gastric juice (*Helicobacter Pylori* (HP) and other microorganisms), as for biopsy materials, we studied micro flora and urease activity (methods developed previously). Isolated microorganisms were identified by generally accepted methods; as for their sensitivity to antimicrobial drugs it was tested by disco-diffuse method. HP was cultivated from gastric juices in 57.5%, from biopsy material from cardia in 45.0% and esophagus (25.0%). Presence of antibodies to HP in the majority of samples (63.3%) is an evidence of HP persistence causes its respective reaction. Study of gastric juices acidity showed only 22.5% of patients had normal secretion (5-10 mmol/hour); there was decreased gastric secretion in 77.5%. Cultivation assay of the mentioned microorganisms depending on gastric juice acidity level was particularly interesting. This assay showed tendency of HP exposure while acidity increasing and reverse tendency related to *Candidae*. This tendency is especially obvious will testing gastric juices for HP and *Candidae*. High frequency of HP in patients with LC might cause by various common and local factors: total reduce of resistance to infection in patients with LC, poor blood circulation in portal system resulted in local decrease of mucosa resistance in the gastroduodenal zone. At the same time, HP invasion worsens all the processes described above due to progressive mucosa atrophy and development of hypo- and achlorhydria. Different strains of HP differ by their virulence, i.e. ability for adhesion on GM and, probably, esophagus, and some other pathogenic features that allow HP to affect tissues of microorganism resulting bleeding and other complications. Pathogenesis of "hepatogenous" erosive-ulcerous process in the stomach of patients with LC and PH lasts for a long time with significant atrophy of the mucosa and, respectively, low secretor activity, accompanied by HP worsens course of the main disease and promote esophageal-gastric bleeding.

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ORIGINAL ANTIBACTERIAL TECHNOLOGY IN THE PROPHYLAXIS AND COMPLEX TREATMENT OF PURULENT-INFLAMMATORY DISEASES OF THE LUNGS AT PATIENTS WITH LONG-TERM OF ARTIFICIAL VENTILATION OF LUNG

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Artificial ventilation of lungs (AVL), improved technologies and methods of invasive respiratory support led to good results on intensive care of critical conditions. Nowadays the risks of developing of ventilator-associated pneumonia, angiogenic sepsis, polyorganic insufficiency etc. are major concerns The purpose of study was to investigate activity of the new antibacterial drug called "FarGALS" for prevention and treatment of pyo-inflammatory lung disease in patients being long-term AVL. By the results of bacteriologic tests from trachea, mouth, and swabs from bronchi of patient with AVL in 2008 patient were divided in 2 groups: 1st - patients administered FarGALS (1:4); 2nd - patient with traditional treatment. One hundred fifty samples were cultured with following antibiotic sensitivity test of isolates by disk diffusion method; 66 (43.0%) were culture positive, including gram positive (14.0%), gram negative (65.0%) and fungus (21.0%); 87 (57.0%) were culture negative. Antibiotic sensitivity test revealed resistance to meropenem (9.7% resistant strains), polymixin B (10.0%), ofloxacin (39.0%) and amikacin (42.0%); in gram-negative bacteria, the resistance observed for penicillin. Antifungal resistance pattern revealed Candida resistant to Citeal (12.5% resistance strain), brilliant [ethyl] green (17.0%), nistatin (22.0%), nitrocsolin (22.0%), terbinaphen (33.0%), amphotericin (34.0%) and fluconasole (43.0%). Analysis of the antimicrobial activity of FarGALS shows high sensitive to all culture. Thereby, in the patient on long-term AVL we observed tendency prevail of gram negative micro flora. This flora was resistance to common antibiotics and clinical improvement observed on 2-3 day after administration of FarGALS. The clinical improvement observed on 5-6 day while traditional treatment resulted in improvement on 8-10 days. High activity of the drug to polyresistance strain allows continuing further investigations in patients with long-term AVL.

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EFFECTIVITY OF DIGESTIVE ORGANS CANDIDIASIS THERAPY BY INDIVIDUAL TESTING DOSES OF NYSTATINE

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In the current study the efficiency therapy of Candidiasis of digestive organs by individually tested doses of Nystatin determined during the process of non- invasive express- method of drug testing according to R.Voll were assessed using the usual laboratory analysis. The research has been carried out in the group of 55 patients. The main group of 32 patients (12 male, 20 female) were treated with tested doses of Nystatine determining during the process of "drug testing" according to R.Voll. The control group consisted of 23 patients (8 male, 15 female) had received

Nystatin by generally recommended doses according the routine protocol. The usual laboratory analysis consisted of microscopic and cultural study of the material scraped from the mucous membrane of the oropharynx, feces culture. In addition, the microscopic study in the hystologic material, the bacteriologic examination of cultures from the pharynx and feces were conducted. Testing of singular and daily doses of Nystatin determined during the process of non- invasive express- method of "drug testig" according to R. Voll. On the patients with different forms of Candidiasis of digestive organs: oropharyngeal Candidiasis, Candidiasis of the esophagus, gastric candidiasis, candidiasis of the intestines the therapy of tested doses of Nystatin, in a comparison with control group, was more effective. The whole course dose of Nystatin in the therapy of candidiasis of digestive organs was less than the total dose recommended in the traditional therapy.

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AN ON-SITE SURVEY OF YELLOW FEVER VACCINATION CLINIC PRACTICES, PENNSYLVANIA, USA, 2008

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As part of a larger survey of yellow fever (YF) vaccination practices in Pennsylvania (PA), we conducted site visits at selected YF vaccination clinics in the state. This was the first evaluation of the YF vaccination program in the United States. The objective was to assess the practices of YF vaccination clinics to better inform guidance to providers. YF clinics in PA were identified from the national YF vaccination clinic registry. A geographically representative sample of all registered YF PA clinics was selected for site visits by using a Stratified Random Sampling plan with Probability Proportional to Size (PPS). Designated providers at the facilities selected for visits were contacted in advance, and participation was voluntary. Data collected included information on clinic setting, staffing, staff knowledge, and vaccine administration and storage practices. We visited 21 (13%) of 165 YF clinics in PA. The types of clinics visited included employee health (24%), specialty medical practice (24%), primary care medical practice (19%), corporate travel medicine (14%), and university health centers or other (19%). Of the clinics visited, 86% had a currently practicing stamp owner on site, 90% maintained a supply of International Certificates of Vaccination and Prophylaxis, 91% gave Vaccine Information Statements to all patients, and 86% kept a log of temperatures for the refrigerator used to store YF vaccine. However, only 57% of clinics kept a separate log of YF vaccine doses administered. In conclusion, site visits to this sample of PA YF clinics showed that adherence to many CDC-recommended vaccination practices are high. However, practices should be improved in some areas. Since practices at these clinics are likely representative of those throughout the country, this survey shows the need for educational tools to optimize YF vaccine administration in the United States. CDC is developing an online education module to be distributed to the state health departments for use by their YF vaccine providers.

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KETAMINE METABOLISM AND TEST ON EXIT PERSONS SUITABILITY DISCUSSION

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This study was undertaken to discuss if the Ketamine test can be a suitable test item for exit persons. We analyzed Ketamine metabolism process in body and compare the test methods of Ketamine and its metabolites. Our results suggested that Ketamine metabolism is fast, and its metabolic half-life is short. Many test methods are being used. But rapid test has time limit for sampling, and sensitivity is very low comparing to GC-MS. In conclusion, at present, there is no suitable test method with good operability for exit persons.

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PREVALENCE OF CHAGAS DISEASE IN U.S. LATIN AMERICAN IMMIGRANT POPULATION WITH CARDIOMYOPATHY

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Chagas disease is the leading cause of cardiomyopathy in Latin America and is believed to infect 16-18 million people. Given recent immigration trends in the United States, there is a large population at risk. However, no data exists regarding the prevalence of Chagas cardiomyopathy in the US. This study aims to determine the prevalence of Chagas disease in a US population of Latin American immigrants with cardiomyopathy. 93 Latin American immigrant patients with idiopathic cardiomyopathy were enrolled from the cardiology clinic at a Los Angeles county hospital. Screening for Chagas disease was performed with both an immunoflourescense assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Testing was performed by the Center for Disease Control and Prevention (CDC). The mean age of patients was 55.6 years and had lived in the US for an average of 22.6 years. Mean ejection fraction was 24.6% and mean left ventricular end-diastolic diameter (LVEDD) was 6.46cm. Countries of origin were as follows: México 51, El Salvador 26, Guatemala 11, Nicaragua 2, Honduras 2, Argentina 1. Fifteen patients were positive by both IFA and ELISA (16.1%). Mean age of positive patients was 61.8 years with mean ejection fraction of 18% and left ventricular end-diastolic diameter of 6.7 cm. The mean time of residence in the US was 16.6 years. 7 patients were from El Salvador (26.9%), 5 from Mexico (9.8%), and 3 from Guatemala (27.3%). Our results demonstrate that Chagas disease is a common cause of cardiomyopathy in US Latin American immigrants, accounting for 16.1% of cases in this population. It is important to identify it as the etiology as both prognosis and management may differ in comparison to other etiologies of cardiomyopathy. In addition, disease progression may be altered by early recognition and treatment prior to onset of clinically manifest heart failure.

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CLINICAL FACTORS PREDICTIVE OF ENCEPHALITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS*

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Angiostrongyliasis caused by *Angiostrongylus cantonensis*, a human parasitic disease in tropical areas, is currently a global health problem due to extensive international travel and eating habits. Most affected individuals develop eosinophilic meningitis, while a minority of patients develop encephalitic angiostrongyliasis (EA), which is fatal in most cases. The risk factors for EA development currently are unclear. We conducted a case-control study in a hospital situated in an endemic area of Thailand. Adults clinically diagnosed with angiostrongyliasis who developed encephalitis and unmatched controls with angiostrongyliasis and meningitis were included for analysis. Logistic regression analysis was used to assess the variables predictive of encephalitis, including exposure history, clinical signs and symptoms, and laboratory data. In total, 14 patients and 80 controls were enrolled for the study. Age (adjusted odds ratio [OR], 1.22; 95% confidence interval [CI], 1.05-1.42), duration of headache (adjusted OR, 1.26; 95% CI 1.03-1.55), and fever greater than 38.0°C (adjusted OR, 37.05; 95% CI 1.59-862.35) were identified as statistically significant factors for EA prediction. In conclusion, elderly

patients with angiostrongyliasis experiencing fever and prolonged headaches were at the highest risk of developing encephalitis. Thus, timely identification, and appropriate evaluation and treatment of suspected cases of angiostrongyliasis may aid in preventing the development of EA.

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PREVALENCE OF CHAGAS DISEASE IN U.S. LATIN IMMIGRANT POPULATION WITH CONDUCTION ABNORMALITIES ON ELECTROCARDIOGRAM

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Chagas disease is the leading cause of cardiomyopathy in Latin America and is believed to infect 16-18 million people. Given recent immigration trends in the United States, there is a large population at risk. Early stage cardiac involvement usually presents with conduction abnormalities on electrocardiogram (EKG), including right bundle branch block (RBBB), left anterior and posterior fascicular blocks (LAFB/LPFB), and left bundle branch block (LBBB). Identification of disease at this stage may lead to early treatment and potentially delay the progression to end-stage cardiomyopathy. This study aims to determine the prevalence of Chagas disease in a US population of Latin American immigrants with conduction abnormalities on EKG. All EKGs performed in a Los Angeles County hospital and clinic system were screened for presence of RBBB, LAFB, LPFB, or LBBB. Patients were contacted and enrolled in the trial if they resided in Latin America for at least 12 months and had no history of cardiac disease. 238 consecutive patients were screened for Chagas disease was performed with both an immunoflourescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Testing was performed by the Center for Disease Control and Prevention (CDC). The mean age of patients was 47.6 years and had lived in the US for an average of 20.15 years. 32.7% of patients had isolated RBBB, 43.7% LAFB, 7.2% LBBB, 7.2% RBBB/LAFB, and 8.8% RBBB/LPFB. Countries of origin were as follows: Mexico 146, El Salvador 56, Guatemala 19, Nicaragua 4, Honduras 5, Argentina 4, Peru 2, Costa Rica 2, and Bolivia 1. Eleven patients were positive by both IFA and ELISA (4.6%). 6 patients were from El Salvador (10.7%), 3 from Mexico (2.1%), 1 from Honduras (20%), and 1 from Argentina (25%). 4 patients had RBBB (5.1%), 4 LAFB (3.8%), 2 RBBB/LAFB (11.8%), and 1 RBBB/LPFB (4.8%). No positive patients had LBBB. One patient was positive by IFA and negative by ELISA with LAFB. Our study demonstrates a significant prevalence of Chagas disease in Latin American immigrants with conduction abnormalities on EKG. Evidence of conduction abnormalities in Latin American immigrants without explanation should prompt consideration of Chagas disease as the etiology.

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COST-EFFECTIVENESS OF INFLUENZA IMMUNIZATION IN ADULT CANCER PATIENTS IN TAIWAN

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The aim of this study was to investigate the efficacy of the influenza vaccine among cancer patients in Taiwan. We determined the effect of immunization on the following outcomes of disease: hospitalizations, emergency department visits, hospital outpatient visits, physician office visits, and deaths. Cost-effectiveness was analyzed from the prospective of the health care system and society. A decision tree used estimated of disease burden and costs based on data from published and unpublished sources. The model followed 34,112 cancer patients aged 20-64 years who were followed by the Taiwan National Cancer Registry in 2002. An influenza immunization program for the cancer population would prevent 2,555 cases of all types of influenza infection, 660 of which would be serious cases involving hospitalization, emergency department visits and death. From the perspective of the health care system, the program would cost US$7.7 million, providing a net savings of US$5.4 million. From a societal perspective, the program would cost US$28.6 million, providing a net savings of US$22.3 million. Compared with the no vaccination strategy, the vaccination program resulted in a savings of US$2107 and US$6,338 per case averted from health care and societal perspective, respectively, and 110 lives saved. Lesser disease burden, greater vaccine efficacy and lower cost of hospitalizations increased cost-effectiveness. The influenza immunization for cancer patients is cost-saving and cost-effective from a health care and societal perspective in Taiwan. We highly recommend annual influenza vaccinations for this patient group.

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DEVELOPMENT OF A QUANTITATITVE REAL-TIME PCR (QPCR) ASSAY FOR *RICKETTSIA PARKERI*

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Rickettsia parkeri, a spotted fever group rickettsia, has recently been found to be pathogenic to humans causing an eschar-associated relatively mild febrile illness. *R. parkeri* rickettsiosis has been misdiagnosed previously as Rocky Mountain spotted fever (RMSF) and rickettsialpox during the past decades due to serologic cross reactivity and lack of species-specific diagnostic methods. A quantitative real-time polymerase chain reaction (qPCR) assay has been developed and optimized to detect *R. parkeri* by targeting a 96 bp fragment of the outer membrane protein B gene (*ompB*) using a species-specific molecular beacon probe. This qPCR assay does not detect nucleic acid from other rickettsiae including: *R. rickettsii*, *R. conorii*, *R. montanensis, R. slovaka, R. sibirica, R. akari, R. japonica, R. prowazekii*, *R typhi*, or *R. canadensis*. Moreover, the assay does not react with nucleic acid preparations from other closely and distantly related bacteria: *Orientia tsutsugamushi*, *Neorickettsia risticii*, *Neorickettsia sennetsu*, *Franciscella persica*, *Bartonella quintana*, *Bartonella vinsonii, Legionella pneumophila*, *Proteus mirabilis*, *Salmonella enterica*, *Escherichia coli* or *Staphylococcus aureus*. The limit-of-detection for the assay was found to be 3 copies per reaction. This sensitive and specific assay is capable of detecting *R. parkeri* nucleic acid in tick vector, animal host and human clinical and epidemiological samples.

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INABILITY OF *AMBLYOMMA AMERICANUM* LARVAE TO ACQUIRE *EHRLICHIA CHAFFEENSIS* FROM AN INFECTED DOG

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A cross-breed beagle dog was inoculated subcutaneously with the MO-1 isolate of *Ehrlichia chaffeensis* in its first passage in DH82 cell culture. The 1 ml inoculum contained 10^6 infected cells. Dog exhibited mild fever on day 28-33 post-inoculation, which was the only clinical symptom observed. Blood and serum samples were collected twice weekly for culturing, PCR, and IFA during the 2-month observation period. Uninfected *A. americanum* larvae were placed upon the dog on days 10, 17, and 24 post-inoculation for acquisition feeding. *Ehrlichia chaffeensis* was successfully reisolated from the dog's blood from day 5 to day 22 post-inoculation. The dog seroconverted by day 8 post-inoculation and remained seropositive for the duration of the study. However, all blood-PCR results were negative, and ehrlichial DNA was not detected in the acquisition-fed ticks tested as either engorged larvae or freshly molted nymphs. The remaining nymphs were fed upon a naïve dog, which neither seroconverted nor exhibited any clinical signs of ehrlichial infection during

the following 2 months of observation. Thus, the dog was susceptible to infection with low-passage *E. chaffeensis* introduced via the subcutaneous route, but the resulting level of bacteremia was apparently insufficient for transmission of the agent to larval *A. americanum* ticks.

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GENETIC RELATIONSHPS OF 364D AND HLP#2 SEROTYPES TO *RICKETTSIA RICKETTSII*

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Complete genome sequences for *Rickettsia* serotypes 364D and Hlp#2, and *R. rickettsii* Hino were determined in a CDC-JGI/LANL collaborative effort. The unique and conserved features of these new genome sequences were compared to the complete genome sequences of *R. rickettsii* Sheila Smith (SS) and Iowa. Both 364D and Hlp#2 serotypes have unique biological and antigenic features from those of most isolates of *R. rickettsii* but whether these differences warranted their placement in different species or subspecies was unclear. DNAs were extracted from rickettsiae grown in Vero or irradiated L929 cells and purified by Renografin density gradient centrifugation. Shotgun Sanger, Illumina, and/or 454 pyrosequencing protocols were used. Sequence comparisons and annotations were made with MAVID, PipMaker, ORF finding programs (Glimmer, GeneMark, GetORF), REPUTER, tRNAscan-SE, BLAST and ClustalW. Three additional 364D serotype-like isolates were obtained in Vero cells from *Dermacentor occidentalis* ticks collected in different sites in Southern California. The genome sequence of 364D was readily distinguished from Hlp#2 and the three isolates of *R. rickettsii* by the presence of an inverted 50 Kb region which has 15 Kb of unique sequence. This region contains *tra* and conjugation protein genes found in *R. bellii* and to a lesser extent in *R. amblyommii*, *R. massiliae* and *R. montanensis*. *R. rickettsii* SS had a unique 10.6 Kb deletion relative to all the other isolates. Hino and Iowa sequences were very similar even though Hino was from a fatal case of Rocky Mountain spotted fever and Iowa is an attenuated vaccine candidate. Forty of the 239 unique 364D INDEL sites were tested and found to be conserved among all four 364D isolates. Hlp#2 had 33 INDELs where it differed from all the other isolates of *R. rickettsii*. In conclusion, *R. rickettsii* is a relatively variable species of *Rickettsia* associated with numerous species of ticks. Data obtained here suggests the Hlp#2 agent should be considered a subspecies of *R. rickettsii* and that *Dermacentor andersoni* associated Montana isolates like SS differ significantly from *D. variabilis* -associated isolates like Hino and Iowa. 364D causes a mild eschar-associated illness in California. It is also genetically distinct enough to be named a new species, *R. philipii*, in honor of Robert and Cornelius Philip, pioneers in the study of rickettsial agents and their tick vectors.

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TEMPORAL PATTERNS OF EARLY CYTOKINE IMMUNE RESPONSE TO INFECTION WITH *BORELLIA BURGDORFERI*

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Early Lyme disease is caused by *Borrelia burgdorferi* infection of the skin. Serologic testing by ELISA is commonly negative in the first weeks of infection. Convalescent serologies are often negative in patients after prompt antibiotic therapy. It is unknown how immune response may vary before, during, and after treatment and how specific biomarkers may relate to clinical serology. Sera were drawn at 4 time points over 4 months of follow-up from 17 patients with early Lyme disease. A total of 30 cytokines were measured using the Luminex bead based system and compared to 12 uninfected controls. Serostatus by ELISA and confirmatory western blot was measured by a commercial lab. Patients with early, untreated Lyme disease had elevated levels of 9 cytokines when compared with controls ($p<.10$), including Eotaxin, IL-12, IL-7, IP-10, IL-IRA, MCP-1, MIP1, MIG and HGF. Following treatment, these differences disappeared for IL-IRA, IP-10 and MIG, while the remaining six cytokines remained significantly elevated across all time points. While not initially elevated, RANTES levels increased post-treatment and remained significantly elevated as well. Seropositivity pre-treatment was associated with elevations in 8 cytokines, including EGF, FGF-β, G-CSF, IFNα, IL-1β, IL-7, MCP-1 and MIP-1α ($p<.10$). Patients with delayed seroconversion during treatment showed increasing levels of 6 of these cytokines over this period, whereas those that remained persistently sero-negative maintained or decreased their levels. Eotaxin levels did not differ significantly by serostatus. Immune response to early Lyme disease may show temporal patterns, including initial elevations of certain cytokines (such as IP-10) as well as later elevations of others (such as RANTES). Eotaxin was significantly elevated compared to controls at all time points but seems unrelated to patient serostatus. Elevation of other cytokines appears to correspond with seroreactivity. The relationship between underlying immune response and clinical outcomes warrants further research.

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MOLECULAR EPIDEMIOLOGY OF POWASSAN VIRUS IN NORTH AMERICA: BAYESIAN ANALYSES REVEAL STABLE POPULATION SIZES THROUGH TIME

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Powassan encephalitis, the illness caused by infection with Powassan virus (POWV), may be an emerging disease. Human cases have increased in frequency over the past ten years, with only 27 human cases reported between 1958 and 1998, and at least 16 human cases reported from 1998 to 2009. POWV is the only tick-borne flavivirus endemic to the western hemisphere, where it is transmitted mainly between Ixodes cookei, and groundhogs (Marmota monax). Deer tick virus (DTV), a genotype of POWV that is frequently isolated from deer ticks (Ix. scapularis), appears to be maintained in an enzootic cycle between deer ticks and white-footed mice (Peromyscus leucopus). DTV has been isolated from ticks in several regions of North America, including the upper Midwest and the eastern seaboard. Maximum likelihood and Bayesian phylogenetic inferences using full and partitioned sequences showed two supported, reciprocally monophyletic lineages corresponding to POWV and DTV. Bayesian skyline plots based on year-of-sampling data indicate no significant population size change for both virus lineages. Statistical model-based selection analyses showed overwhelming evidence of purifying selection in both lineages. Positive selection was found in NS5 sequences for both lineages, and envelope sequences for POWV. Collectively, our findings support strong genetic conservation among POWV and DTV.

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DISTRIBUTION OF SPOTTED FEVER-GROUP *RICKETTSIAE* IN CANINES FROM TENNESSEE

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Rocky Mountain spotted fever (RMSF) is an important tick-borne disease throughout the southeastern United States and the most common tick-borne infection in Tennessee. RMSF is caused by *Rickettsia rickettsii*,

a member of the spotted fever-group (SFG), and transmitted by *Dermacentor variabilis* ticks. Since 1990, RMSF incidence in Tennessee has increased making Tennessee one of the highest reporting states. In Tennessee a gradient is seen with increasing incidence from east to west. The reasons for this disease gradient remain elusive at this point. Domestic canines may be used as sentinels to assess geographic foci of RMSF. Additionally, dogs may play an important role in human RMSF as potential carriers of rickettsiae-infected ticks. This study seeks to assess the prevalence of SFGR among dogs and relate canine prevalence to human RMSF cases. A survey was conducted to assess the seroprevalence of antibodies to spotted fever-group rickettsiae (SFGR) among canines throughout the states of Tennessee. Serum samples were collected from 860 dogs and antibodies to SFGR were detected using enzyme immunoassays (EIA). Samples were initially screened to identify *Rickettsia* and subsequently tested for antibodies to *R. rickettsii*, *R. montana*, and *R. amblyomii*. Preliminary data suggests that exposure to *Rickettsia* at the county level is 3–64%. Similar to RMSF human cases, the Ridge and Valley ecoregion in east Tennessee has the lowest exposure to *Rickettsia* in canines. Additionally, Cheatham, Henderson, Henry were found to be "hot spots" for canine exposure as they are for human RMSF incidence. Our data indicate that *Rickettsia* exposure in domestic canines is wide-spread throughout the state of Tennessee.

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EFFECTS OF FLEA FEEDING ON EARLY INNATE IMMUNE EVENTS IN THE SKIN AND TRANSMISSION OF *YERSINIA PESTIS*

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Transmission of pathogens by arthropod vectors is known to affect host responses when compared to needle-injection. This phenomenon has not been well studied in the transmission of *Yersinia pestis* by fleas. We have characterized an intradermal model of *Y. pestis* infection in mouse ears. We used this model to compare early innate immune events in ear tissue after infection. Transmission of *Y. pestis* by the flea vector *Xenopsylla cheopis* is unpredictable, and there is a large amount of variability in the dose of bacteria delivered by flea bite. Therefore in this study we allowed adult fleas to bloodfeed on mouse ears, and then immediately afterward infected mice intradermally in the same ear with ~250pfu of virulent *Y. pestis* strain 195/P. Mice infected with *Y. pestis* by intradermal needle inoculation alone and mice exposed to fleas only served as controls. Paired ear and draining lymph node samples were taken at 3, 6, 12, and 24 hours post infection. Skin and lymph node cells were analyzed by flow cytometry to measure the nature and dynamics of the inflammatory response. Bacterial loads at each timepoint were measured in the ear, draining lymph node, spleen, and blood. This allowed us to evaluate the effect of the presence of flea saliva at the time of infection with a known dose of *Y. pestis*.

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INFECTION RATES OF THE TRIATOMINE BUG *TRIATOMA RUBIDA* WITH *TRYPANOSOMA CRUZI*, THE CAUSATIVE AGENT OF CHAGAS DISEASE, IN THE TUCSON AREA OF ARIZONA

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Arizona has the most human-triatomine bug contacts in the USA (American Association of Poison Control Centers; Arizona Poison and Drug Information Center, University of Arizona Health Sciences Center). *Triatoma rubida* is one of the most common "invading bugs" and is strongly associated with the pack rat *Neotoma albigula*. As humans move to the suburban areas of Tucson and invade the pack rat habitats, these contacts are becoming more frequent. To begin to assess the threat of Chagas transmission from these vectors, we analyzed 164 bugs found in and around houses for the presence of *Trypansoma cruzi*. These bugs were collected in 22 sites from May to December 2006 in the Tucson area, with over 60% of the adults collected during a two week period in late May and early June. 41.5% of the bugs were positive for *T. cruzi* and at least one infected bug was found at each collection site. Although no autochthonous cases have been reported in Arizona, the risk for transmission may be higher than previously thought.

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GEOGRAPHICAL ASSESSMENT OF RICKETTSIAL INFECTIONS IN RODENTS IN INDONESIA

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Rickettsia are an emerging bacterial pathogen in Southeast Asia and their ability to cause significant human disease has been documented in this region. Indonesia is the world's largest archipelago with over 17,000 islands and 234 million residents. Murine typhus is endemic across Indonesia, while spotted fever group rickettsiae have been reported in the Gag islands, and residents of East Java have demonstrated evidence of infections from Orientia tsutsugamushi. In order to determine the primary zoonotic reservoirs for disease in humans, rodents were trapped on the islands of Java, Sumatra, Sulawesi and Kalimantan. Serum, organs and ectoparasites were collected from each rodent. Infection was determined by indirect ELISA and the presence of Rickettsia in serum and ectoparisites was detected by PCR. A total of 491 rodents were trapped including shrews, mice and eight species of rats. The most common trapped rodent was Rattus tanezumi. Several types of ectoparasites were collected including chiggers, fleas, lice, mites and ticks. The overall seroprevalence of rickettsial infections in rats was 50% with 26% spotted fever group (SFG), 14% typhus group (TG) and 10% scrub typhus group (STG). The highest overall seroprevalence of rickettsial infections (81%) was found in Java where there was a 61% seroprevalence of TG, 20% seroprevalence of SFG and 0% seroprevalence of STG infections. Serology results revealed R. tanezumi as the main reservoir for TG with PCR results from organs and Xenopsylla cheopis collected from this species of rodent confirming the presence of R. typhii. The seroprevalence of rickettsial infections in rats from Kalimantan, Sumatra and Sulawesi were 76%, 35% and 28% respectively. Further analyses to determine the primary reservoirs and types of Rickettsia on these islands are in progress. Our current results suggest that the risk for rickettsial infection is high in many areas in Indonesia, although the rodent reservoirs and type vary.

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AFRICAN TICK-BITE FEVER IN A TAIWANESE TRAVELER RETURNING FROM SOUTH AFRICA: MOLECULAR AND SEROLOGICAL STUDIES

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We report the first imported case of African tick bite fever (ATBF) in a patient from Taiwan who returned from a 10-day trip to South Africa. Diagnosis was confirmed by polymerase chain reaction (PCR) from eschar biopsies. Portions of rickettsial *ompA* (491 bp) and *ompB* (273bp) genes were amplified and subsequent sequencing of PCR product showed its 100% identity with *R. africae*. Microimmunofluorescence (MIF) assay of

patient's serum on days 14 and 46 after the onset of illness revealed IgG seroconversion when tested with spotted fever group (SFG) rickettsiae antigens, including *R. africae*. The patient clinically improved on third day of the 14 days treatment with combination of ciprofloxacin and minocycline. Based on the patient's travel history and chronology of clinical symptoms, we strongly suspect that the tick-biting event occurred in Kruger National Park.

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ANTIBODY DETERMINANTS OF PROTECTION AND ENHANCEMENT OF SECONDARY DENGUE VIRUS INFECTION *IN VITRO* AND *IN VIVO*

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A mouse model of dengue disease is of critical importance to furthering the understanding of dengue pathogenesis. Previously, our group demonstrated that interferon-α, β, and -γ receptor-deficient (AG129) mice are susceptible to all four strains of dengue virus (DENV), develop viremia and viral load in relevant tissues, and succumb to a vascular leak syndrome when given a lethal infection of a mouse-adapted DENV2 strain, D2S10. We have used the AG129 mouse to model protection against DENV infection in both passive transfer and sequential infection studies, and have recently characterized conditions that support antibody-dependent enhancement (ADE) of DENV infection using passive transfer of either anti-DENV sera or monoclonal antibodies (MAb) followed by sub-lethal infection with DENV-2 D2S10. Here, we describe ADE of two clinical DENV-1 and DENV-2 strains. Specifically, MAb or anti-DENV sera transferred prior to infection with either strain results in significantly enhanced viral burden in sera, small intestine and liver as compared to controls. While many studies have characterized anti-DENV MAbs *in vitro,* few studies have attempted to systematically measure the relationship between *in vitro* correlates of *in vivo* outcome. Using our well-characterized AG129 model, studies are underway to define Ab determinants crucial to immune protection and enhancement of DENV infection at the serotype-, domain- and epitope-specific levels. First, the role of serotype-specific versus serotype cross-reactive anti-DENV sera in mediating neutralization and enhancement of DENV infection *in vitro* and *in vivo* (monitoring viral load and survival) is being assessed. Next, we are evaluating the role of envelope domain III (EDIII)-specific Abs by determining the *in vivo* effect of depleting EDIII-specific Abs from mouse serum in our passive transfer model of DENV infection. Finally, using both *in vitro* neutralization and enhancement assays and *in vivo* challenge studies, we are characterizing a panel of mouse and human MAbs directed against multiple DENV E and prM/M epitopes that are both serotype-specific and cross-reactive in nature. Taken together, this data allows us to address the complex role of anti-DENV antibodies in both protection and enhancement, which has direct relevance to the development of tetravalent dengue vaccines.

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DIFFERENCES IN CLINICAL PRESENTATION AND RELATION TO IMMUNE STATUS AMONG DENGUE VIRUS SEROTYPES IN A HOSPITAL-BASED STUDY IN NICARAGUA

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Dengue is a mosquito-borne disease caused by four distinct dengue virus serotypes (DENV1-4). DENV infection causes a range of disease, from dengue fever to the potentially fatal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). To perform a detailed investigation of the differences in dengue caused by distinct serotypes, an observational study of 337 patients from 6 months to 14 years of age with laboratory-confirmed dengue was conducted between July 2005 and January 2009 in the National Pediatric Reference Hospital in Managua, Nicaragua. The majority of subjects (82%) were hospitalized, while the rest were followed as outpatients. Of the 309 (89%) cases in which serotype was identified, DENV-2 predominated (51%), followed by DENV-3 (37%) and DENV-1 (12%). For the first 3 years, DENV-2 was responsible for 85% of cases, while in the fourth year, DENV-3 caused 74% of cases, and DENV-1 accounted for 5%-18% of cases annually. DENV-2 was more common in 10-14 year-olds ($p = 0.004$) while DENV-3 predominated in 1-4 year-olds ($p = 0.006$). Likewise, the vast majority of DENV-2 cases displayed a secondary immune response (86%) compared to DENV-1 and DENV-3 cases, which occurred to a much greater degree in primary infections (41% and 44% secondary infections, respectively; $p<0.0001$). Only in the case of DENV-2 was secondary infection significantly associated with DHF/DSS (OR = 6.80, 95% CI = 1.45-32.98; $p = 0.014$), though the other serotypes trended towards significance; because infants develop DHF/DSS in primary infections, they were excluded from this analysis. In terms of severity, DENV-2 was significantly associated with DHF/DSS (52% were DHF/DSS vs. 19% of DENV-1 cases and 30% of DENV-3 cases, $p<0.0001$ vs. DENV-2) as well as with more severe clinical manifestations (plasma leakage, shock, significant bleeding, internal bleeding, or alteration of the central nervous system; $p<0.0001$). However, DENV-1 was more likely to present signs of shock without the hemorrhagic symptoms and thrombocytopenia that define DSS according to the World Health Organization (OR = 4.11, 95% CI = 1.32-12.81, $p = 0.023$). DENV-2 caused more cases of DHF/DSS in 2006-2008 than in 2005 ($p<0.0001$), which coincides with a DENV-2 clade replacement in 2006 that has been associated with greater disease severity. These results demonstrate that there are significant differences in clinical presentation and the relation to immune status between DENV serotypes.

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PHYLOGEOGRAPHY AND MOLECULAR EVOLUTION OF DENGUE VIRUS TYPE 1 IN PUERTO RICO, 1981-1998

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Dengue is the most important mosquito-borne virus in the world, with the World Health Organization estimating that more than 2.5 billion people are at risk. Dengue consists of a group of four closely related serotypes, DENV-1 - 4, which cause a wide spectrum of disease, from mild dengue fever (DF) to more severe dengue hemorrhagic fever (DHF) and shock syndrome (DSS). All four serotypes have expanded geographically in the past 30 years and currently co-circulate in most tropical regions of the

world (hyperendemicity), including Puerto Rico. DENV-1 was introduced and became endemic in 1977, causing a large epidemic in 1978 and a smaller outbreak in 1981. 33 whole genome DENV-1 isolates were sequenced from Puerto Rico from 1981-1998, along with 17 envelope genes from other parts of the Caribbean during the same time frame. The Puerto Rico samples all fall into the American genotype and DENV-1 appears to be time structured with lineage turnover occurring, similar to previous studies on DENV-2 and DENV-4 in Puerto Rico. Phylogenetic analyses shows Puerto Rico isolates falling into two clades, the newer clade replacing the older clade over time. The replacement clade evolved locally rather than being introduced, and is separated by one amino acid change in the premembrane, three in the envelope, one in NS3, and three in NS5. This suggests Puerto Rico serves as a location in the Caribbean for exporting dengue virus, as well as importing on a smaller scale.

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TRANSFORMING MODELS INTO USER-FRIENDLY PROGRAMS FOR EVALUATING DISEASE CONTROL STRATEGIES AT THE LOCAL SCALE

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Although models have the potential to improve our ability to predict disease dynamics and identify more effective control and management strategies, there remains a substantial disconnect between the development of models (typically by scientists) and their use in developing disease management strategies (by e.g., public health officials). Models can be complex and difficult to understand, fail to facilitate development of *local* control strategies, or are not tested with enough rigor to confidently support the development of public health strategies. The objectives of this study were to: (1) transform an existing dengue simulation model into a windows-based user-friendly program that public health officials can use to evaluate different control strategies for a specific location and (2) perform rigorous tests to ensure model accuracy. We will illustrate issues that must be considered when developing models to inform disease management, demonstrate how we addressed these issues with our revised model, and present sensitivity analysis results. Improvements that increased usability include developing a windows interface, creating an entomological survey data sheet to facilitate data collection and entry, and automating processes that were originally executed by the user. Results of the sensitivity analysis identified several parameters with little influence on model output that can be fixed in the model. Other parameters had a large effect on model output, including those associated with density dependence in the larval stage. Slight changes in these parameters generated substantial changes in the mean, minimum, and maximum number of mosquito eggs, larvae, pupae, and adults. Broadly, our results help identify where future empirical studies should be targeted to improve model accuracy and overall knowledge of dengue transmission, illustrate one way that the gap between basic and applied disease ecology can be bridged, and argue for a more direct link between disease models and on-the-ground disease management.

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DENGUE VIRUS SEROPREVALENCE AND SEROINCIDENCE AMONG KENYAN CHILDREN

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Epidemics of dengue fever caused by four distinct serotypes have been documented in many African countries over the past several decades, however little is known about the seroprevalence or incidence of dengue virus infection on the African continent in the absence of an outbreak. A seroprevalence of dengue infection ranging from 1-15% has been documented in eastern coastal regions of Kenya. No studies have analyzed the presence of transmission in western Kenya to date, although potential dengue vectors of the genus *Aedes* are known to exist. This study attempted to describe the seroincidence and seroprevalence of dengue infection among a population of children in western Kenya. A total of 354 samples were obtained from banked serum of healthy afebrile children ages 12 to 47 months from the local area surrounding the Walter Reed Project Kombewa Clinic in the village of Kombam, in Kombewa Division, Kisumu District, Nyanza Province, Kenya. This serum was previously collected from a USAMRU-K malaria vaccine trial between March 2005 and April 2007. An immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) manufactured by Panbio Diagnostics was utilized to detect antibody to an antigen common to all four dengue virus serotypes. The seroprevalence of dengue infection in this population was determined from these 354 samples. Of those samples testing positive, a second serum sample (previously collected from the above malaria vaccine trial) at approximately one year prior was analyzed for dengue virus IgG to determine seroincidence. Our analysis found a seroprevalence of prior dengue virus infection of 1.1% (4 of 354 samples) in this patient population. Further analysis revealed that 1 of the 4 samples was positive one year prior, resulting in an incidence of 8.5 seroconversions per 1000 persons per year. In conclusion, our data reveals that the seroprevalence and incidence of dengue virus in western Kenya appears to be lower than that previously reported in eastern coastal regions of the country. Continued investigation and evaluation of exposure to dengue virus in a different sample population is necessary to further confirm this finding.

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MOLECULAR EPIDEMIOLOGY OF DENV-1 ISOLATED IN MARACAY, VENEZUELA, DURING 1997 - 2007: A PROBABLE CLADE REPLACEMENT EVENT

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A typical pattern of dengue transmission in Maracay, Venezuela, is the co-circulation of multiple DENV serotypes. Within this frame, in the past ten years DENV-1 has had two periods (1997 to 2000 and 2003 to 2008) of high prevalence and one short period (2001 - 2002) in which it was not detected at all. To investigate the possible emergence of new genetic variant(s) associated with the last historic outbreak occurred between June 2006 and March 2008, we performed a phylogenetic analysis of complete genomic sequences of 18 DENV-1 isolated in Maracay from 1997 to 2007 and 15 from worldwide, as well as Envelope (E) gene sequences from DENV-1 isolated in four other Venezuelan states between 1995 and 1997, and from worldwide at different times. It was found that DENV-1 from Maracay grouped in the America-Africa genotype (V) and segregated into two probable clades: one containing the majority of virus isolated before 1999, and another composed of isolates obtained from 1999 to 2007. The mean ratios of nonsynonimous to synonymous substitutions (dN/dS) in all protein coding genes were very low (range: 0.2 - 0.009). Also, there were a total of 67 aminoacid substitutions being the more relevant non-conservative changes located on E, NS1, NS2A, NS4B and NS5 protein coding genes. In conclusion, the results suggested that a probable clade replacement event occurred between 1998 and 1999, and that the genetic changes were produced by a purifying selection pressure.

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IMINOSUGAR NB-DNJ DELAYS MORTALITY IN LETHAL MODEL OF DENGUE VIRUS INFECTION IN MICE

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The four serotypes of dengue virus (DV1-4) cause more human illness than any other arbovirus worldwide. DV infection results in dengue fever, an acute febrile illness, or the more severe, life-threatening dengue hemorrhagic fever/dengue shock. Immunity to one of the four DV serotypes can increase disease severity upon subsequent infection with another DV serotype via antibody-dependent enhancement (ADE) that facilitates entry of DV into Fcγ receptor-bearing cells. The DV genome consists of 3 structural (C, capsid; prM/M, membrane, and E, envelope) and 7 nonstructural (NS1-5) proteins, of which M, E, and NS1 are glycosylated. No antivirals or vaccines currently exist, and treatment is supportive in nature. We have developed a robust mouse model of DV infection and disease in mice lacking interferon α/β and γ receptors (AG129) that features many of the hallmarks of severe dengue in humans. Recently, we demonstrated ADE in these mice following passive transfer of anti-DV monoclonal antibodies or polyclonal immune sera. Iminosugars such as deoxynojirimycin (DNJ) and its N-alkylated derivatives are glucose analogues that inhibit host cell ER-resident glucosidases required for folding and maturation of glycoproteins. Iminosugars prevent interaction of many viral glycoproteins with ER chaperones, leading to incorrect glycoprotein folding, oligomerization and assembly of infectious virions. The N-linked glycosylation of the prM/M, E, and NS1 proteins are thought to play a role in viral morphogenesis and secretion. We tested the effect of *N*-butyl-DNJ (NB-DNJ) in our mouse model of ADE-induced lethal disease. Mice received 2ug of the anti-E 4G2 MAb intraperitoneally (ip) and were infected 24 hours later with $1x10^5$ DV2 D2S10 iv. Drug was administered daily or twice daily ip on the day of infection and for 7 days p.i.. NB-DNJ given at 0.2 mg/kg/day protected 3/5 (60%) mice from mortality, while PBS control mice all died by day 5 ($p=0.003$). Three of 5 mice receiving NB-DNJ at a much lower dose (0.0001 mg/kg/day) encapsulated in liposomes were protected from death vs. liposome alone, where all 5 mice died within 5 days ($p=0.003$). In addition, NB-DNJ treatment delayed morbidity in infected mice. In conclusion, glucosidase inhibitors reduce DV-induced disease and mortality *in vivo*, and we are currently evaluating other iminosugars with liposome delivery as promising antiviral therapies.

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DENGUE VIRUS PROTEASE NS2B/NS3 WITH A POTENTIAL INHIBITION OF THE TOLL-LIKE RECEPTOR 3 SIGNALING PATHWAY

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Dengue virus (DENV) is a mosquito-borne flavivirus of worldwide distribution that causes in humans, dengue fever (DF) an acute febrile illness or life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The mechanisms of immune response against dengue virus are not completely understood. Toll-like receptors (TLR) are important in mediating inflammation and immune responses by recognizing pathogen specific ligands. TRL3 recognizes double-stranded RNA (dsRNA) activating the interferon (IFN) signaling pathway, a potent defense mechanism against viruses. However, viruses have developed strategies to avoid this response and the viral products that mediated this resistance are under study. After cytoplasmic nucleocapsid release, DENV RNA translates to a polyprotein that with the activity of host and virus peptidase results in the cleavage of three structural (C, E, prM) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Has been reported that DENV infected cells showed resistance to the antiviral action of IFN, for example, NS4B inhibit the α/β IFN signaling. In other flavivirus, Hepatitis C virus, has been described that the viral serine-protease NS3/4A cause a cleavage of TRIF (Toll-IL-1 receptor domain-containing adaptor inducing IFN-β) the adaptor protein linking TLR3. A sequence alignment of TRIF protein showed possible cleavage sites for DENV protease (NS2B/NS3). TLR3 induced activity of IRF3 was assessed using a luciferase reporter assay in the presence or absence of viral protease. We found that luciferase activity was decrease as effect of viral protease. All these events support our hypothesis that NS2B/NS3 DENV interrupts the TLR3 signaling pathway. This phenomenon could constitute an important mechanism of immune evasion in DENV.

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EFFECT OF FCγRII ISOFORMS ON ANTIBODY DEPENDENT ENHANCEMENT OF DENGUE VIRUS INFECTION

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The Antibody Dependent Enhancement (ADE) phenomenon is hypothesized to contribute to the pathogenesis of complicated dengue virus infection. Fc-γ receptor engagement by dengue immune complexes is linked with enhancement of infection. Our current work focuses on the role of FcγRs in ADE of dengue virus infection in primary human target cells. Our previous data showed that dendritic cells mainly express FcγRII; both FcγRIIa and FcγRIIb isoforms. We showed that in mature human dendritic cells, FcγRIIa largely mediates ADE. Therefore, we aimed to study the contributions of these two isoforms in ADE, especially in light of the inhibitory features of the FcγRIIb isoform and focused on downstream consequences. We generated FcγRIIa or FcγRIIb expressing cell lines by transfecting full-length cDNA of each isoform into non-Fc bearing cells, thereby allowing comparative study of cells including infection rates, viral output and infectivity of released virions. Our preliminary data, using binding and internalization studies, suggests that the FcγRIIa isoform mediates ADE more efficiently than FcγRIIb. We will report our current data set to advance the understanding of the involvement of FcγRII isoforms in ADE.

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COMPARISON OF NEUTRALIZING AND ENHANCING TITERS OF PATIENT AND VACCINEE SERA USING A HIGH-THROUGHPUT DENGUE REPORTER VIRUS DETECTION SYSTEM

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Despite the critical need for a safe and effective Dengue virus (DENV) vaccine, development has been hindered by the lack of reliable, high-throughput tools for measuring antibodies that may be protective or, equally important, infection-enhancing. Here we use a recently developed tool, the Dengue Reporter Virus (DRV), to rapidly monitor human serum for protective and pathogenic anti-DENV antibodies. DRVs have been developed by combining a subgenomic replicon encoding an optical reporter (GFP or luciferase) with structural components from defined strains of DENV. Infection is monitored by expression of the reporter gene, providing an objective output that can be quantified using standard optical detection platforms. DRVs retain the antigenic determinants

of wild-type virions, so can be used to rapidly assess humoral immune responses to all four DENV serotypes. However, they lack the viral machinery required to initiate a productive infection, allowing their use under BSL2 conditions. Importantly, the DRV assay can be fully automated for high throughput screening of sera from large scale vaccine trials or epidemiological surveys. In this study, DRVs corresponding to all four DENV serotypes were used to quantify both neutralizing and enhancing antibody titers in human sera. Sera were analyzed from individuals vaccinated with a live attenuated DENV vaccine and from patients naturally exposed to pathogenic DENV. Comparison of sera neutralization and immune-mediated enhancement of DENV infection allowed a complete profile to be compiled for each individual, documenting the potentially protective and pathogenic humoral immune response against each serotype of DENV within each patient. Furthermore, titers obtained using DRVs were comparable to those derived using the plaque reduction neutralization test (PRNT), a manual technique poorly suited for large scale, high-throughput assays. These experiments validate the DRV as a high-throughput tool for measuring neutralizing and enhancing serum responses, and provide a new opportunity to understand the effects of natural infection and vaccination on large patient populations.

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STUDIES OF VECTOR COMPETENCE IN *AEDES AEGYPTI* WITH COLOMBIAN STRAINS OF DENGUE VIRUS: EVALUATION OF SINGLE AND MIXED INFECTIONS

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The *Aedes aegypti* mosquitoes, has features that allow them to be effective vectors of dengue virus (DENV) transmission. Vector Competence is one of these characteristics and concerns of vector susceptibility to infection, and its ability to transmit the virus. Strains belonging to four serotypes of the DENV co-circulating in Colombia, so the objective of this study was to evaluate the vectorial competence of *A. aegypti* in two different strains of serotypes 2 and 3 of DENV in singles and mixed infections. To test this strains 3986/07 (DENV-2) and 3832/06 (DENV-3) were amplified in C6/36 and quantified by RT-qPCR. *A. aegypti* Mosquitoes were challenged orally using artificial feeder. Inocula (2x108 copies/mL) consisted of two strains of the virus mixed with blood by an individual, or a mixture of the two strains. Several days post-feeding were carried out dissections for mosquito's bodies to measure the viral genome by RT-qPCR and viral antigen detection by immunofluorescence. Preliminary findings suggest that in single and mixed infections was found that the amount of viral genome in the DENV-2 strain 3986/07 was higher, which could indicate a better replication capability of this strain. *A. aegypti* mosquitoes exhibited differences in replication of two DENV strains suggesting a variation in vector competence. This fact is of great importance for understanding the transmission patterns and movement of viral variants in endemic areas of our country

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MODELING DENGUE CASES IN HEALTH REGIONS OF COSTA RICA USING EL NIÑO SOUTHERN OSCILLATION AND LOCAL VEGETATION DYNAMICS

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Temporal behavior of dengue fever and dengue hemorrhagic fever (DH/DHF) has been associated with climate, which may modulate mosquito vector populations. El Niño Southern Oscillation (ENSO) fluctuations can be related to sea-surface temperatures in the Pacific that influence precipitation and temperature in Latin America. In addition, vegetation dynamics have been associated with DF/DHF at local scales. In this study, vegetation indices from the Moderate Resolution Imaging Spectrometer (MODIS) and Pacific sea-surface temperature anomalies were used to model weekly DF/DHF cases (2003 to 2007) in Costa Rica and its nine Health Regions (HR). Using cross correlation analyses, positive and negative lags were identified, where DF/DHF cases and each independent variable were better correlated. A sinusoid and non-linear least squares model was applied to fit case data for the county and HR using lagged variables. The countywide model, where variables were lagged according to their highest correlation coefficient, had an R^2 of 0.86. Models including either only the positive or only the negative lags of variables had R^2 values of 0.60 and 0.84, respectively. These models were all able to reproduce a major epidemic in 2005. Model performance differed between HR, with R^2 values from 0.41 (a region with few cases and slight wet/dry seasonality) to 0.85 (a region with marked seasonality and >20,000 cases). Results show that climate and vegetation dynamics are good predictors of DF/DHF cases in Costa Rica. The differences in model fits for HR may be due in part to local conditions that can affect transmission such as altitude, temperature, socioeconomic conditions, prevention and control actions, and human behavior. Moreover, these models may be improved further by using other variables like Atlantic sea surface temperatures. Considering that the HR of Costa Rica may represent conditions common to areas of Latin America and the Caribbean, these models may be applicable to various countries and function as an early warning system to predict epidemics in areas affected by dengue.

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DENV-3 GENOTYPE III IS CIRCULATING IN SÃO PAULO STATE, BRAZIL, DURING THE LAST FIVE YEARS, AND IT HAS NOT BEEN ASSOCIATED WITH THE SEVERE PRESENTATIONS OF THE DISEASE

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Dengue viruses (DENV) are the most important arboviruses of public health significance, and consist of four distinct antigenic types (DENV-1 to -4) that show substantial genetic diversity. These viruses usually cause dengue fever (DF), but some patients experience a more severe form of the illness known as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The first reports of DENV-3 cases in Brazil occurred in the year 2000 and after a short period of co-circulation of DENV-1, -2, and -3, DENV-3 spread rapidly throughout the country. Phylogenetic analyses of DENV-3 isolated from all regions of the world have revealed the existence of four to five DENV-3 genotypes. Genotype III of DENV-3 has been the main genotype circulating in Brazil, but recent studies have indicated that genotype I and genotype V are also circulating in some states of Brazil. In order to evaluate DENV-3 genotypes circulating in São Paulo state, we analyzed the NS1 region of DENV-3 isolated from patients presenting with different clinical manifestations of dengue disease in the city of Ribeirão Preto from 2003 through 2008. Nucleotide sequences from 31 viruses were obtained and compared to 105 DENV-3 corresponding sequences retrieved from GenBank. Phylogenetic analysis showed that São Paulo DENV-3 sequences belong to genotype III and that Puerto Rico strains are closely related to South American strains. Even though this genotype has caused DHF/DSS in other countries, there was no association between São Paulo DENV-3 genotypes and DHF/DSS. However, the circulation of different genotypes in Brazil should be continuously evaluated since a new genotype may arise and this fact may have implications with the pathogenic aspects of this disease and with the epidemiological changes of dengue clinical presentations in South America.

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SPATIO-TEMPORAL PATTERN OF DENGUE VIRUS SPREAD IN URBAN CAIRNS, AUSTRALIA

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Dengue has reemerged as a major global public health concern over the last four decades. In North Queensland (NQLD), Australia, the number and size of dengue epidemics has been on the increase since the early 1990's, with most outbreaks originating from viremic travelers from Papua New Guinea (PNG) and Southeast Asia. In the present study we analyzed the spatio-temporal pattern of the large 2003 outbreak that included 383 dengue confirmed cases in the city of Cairns, and resulted in spraying of 1,163 residences with residual insecticides. We quantified the relationship between dengue transmission and distance to the index case (IC), and generated recommendations for city-wide improvement of dengue surveillance and control. When focal spatial statistics (local K-function) were applied, dengue cases were found to be clustered up to 800 m around the IC (a traveler from PNG diagnosed as having dengue-II 49 days after the onset of symptoms). Space-time analysis (Knox test) showed that the disease spread rapidly, generating 15 space-time clusters (comprising 65% of all cases), and that these clusters varied in severity and extent as a function of their distance to the IC. The spatial and temporal pattern of 40% of the clusters located in the periphery suggests they were originated by secondary cases most likely infected in the core area (within 800 m of the IC). In areas susceptible to non-periodic dengue epidemics like NQLD, effective disease prevention and control depends on prompt response to introduced cases. The development of a decision support system for dengue control in NQLD will rely strongly on the incorporation of accurate mapping and analysis capabilities (GIS and space-time statistics) to better assess where and when cases are introduced, and how far from the ICs control actions should be performed.

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INVOLVING A LOCAL COMMUNITY NETWORK WITHIN A LARGE SCALE, TETRAVALENT DENGUE VACCINE EFFICACY TRIAL IN THAILAND

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Evaluating novel technology is always challenging, even more so when it takes place in emerging regions. We describe the practical experience gained while setting up the first large scale, randomized controlled pediatric clinical efficacy trial of sanofi pasteur's tetravalent dengue vaccine candidate. The study involved a community in the Thai province of Ratchaburi, an area highly endemic for dengue. Effective communication was essential to inform and involve the community. The process was three-fold: 1) to ensure health education programs on dengue disease, 2) to inform the study participants and the community about the vaccine and the study process, and 3) to ensure an appropriate interface and flow of communication between the different stakeholders. Investigators from Mahidol University under the auspices of the Thai Ministry of Public Health took the lead: the sponsor provided information on the tetravalent dengue vaccine. The participating children, their parents and families, teachers, healthcare staff and operational stakeholders of the trial as well as public health professionals, regional politicians and the provincial health authorities were all involved in the process. While this represents a significant investment, community involvement can provide a supportive environment for studies when participants are sufficiently informed and understand the reasons and process surrounding the study. This successful collaboration between private and public bodies, non-governmental organizations and the wider community provides valuable lessons to those developing new solutions to public health concerns.

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DEVELOPMENT OF SANOFI PASTEUR'S RECOMBINANT LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE: 2009 UPDATE

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Sanofi Pasteur has lead the development of a tetravalent dengue vaccine candidate. Using innovative recombinant technology, envelope and pre-membrane genes of the YF 17D vaccine virus were replaced with corresponding genes from dengue serotypes 1_4 viruses. The resulting live-attenuated vaccine has been extensively characterized in preclinical, *in vitro* and animal models. A comprehensive in-house Dengue Technology Platform incorporating molecular biology, serology and CMI tools and assays has been set-up in compliance with WHO guidelines to facilitate the development at all stages from preclinical to its eventual use in target pediatric populations in endemic countries. All four dengue vaccine viruses have been shown to be genetically and phenotypically stable and even more attenuated compared to YF 17D in terms of neurovirulence and absence of hepatotropism in preclinical models. Environmental safety testing demonstrated that vaccine viruses were unable to infect mosquitoes, and when artificially recombined with wild-type viruses, were still attenuated compared to the parental viruses addressing theoretical concerns about the use of this vaccine. In parallel, clinical trials in US, Latin America and Asia have shown the induction of long-lasting immunity and cellular responses in the different populations investigated. Up to mid-2009, more than 1100 participants have received at least one dose of sanofi pasteur's tetravalent dengue vaccine. Transient, low-level viremia and strong humoral immune responses were seen following subcutaneous injections of adults with even less frequent viremia seen in children. An efficacy trial began in Thailand at the beginning of 2009 and other phase II trials are on-going worldwide.

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INTERFERENCE IN VIRAL REPLICATION BETWEEN DENGUE SEROTYPES IN CO-INFECTION OF AN INTERFERON DEFICIENT CELL LINE

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The development of a vaccine has been proposed as the most effective means for control of dengue virus. Clinical investigations with empirically attenuated live vaccine candidates have demonstrated that attenuated strains found to elicit neutralizing antibodies in monovalent formulations failed to elicit neutralizing antibodies when combined in bivalent or tetravalent formulations. To assess whether this effect could involve direct interaction at the cellular level, we investigated whether co-infections with multiple serotypes of dengue had different kinetics of replication as compared to single infection. We utilized a monkey kidney cell line (Vero) completely deficient in interferon production to minimize variations in the host antiviral response. Vero cells were seeded in parallel and then infected with prototype dengue in single or mixed infections at various moities of infection. Samples of supernatant were taken every 24 hours. After extraction of RNA from supernatant, viral RNA was quantified using a validated, serotype-specific q-RT PCR assay. Four days post-infection, we assessed the percentage of live cells using a trypan blue exclusion test and cellular metabolism using a resazurin reduction assay. Statistically significant decreases in the quantity of viral RNA produced was observed as early as 48 hours, and was observed in all mixed infections. The magnitude of the reduction in viral RNA production increased with the amount the competitor virus, but not in a linear fashion. Specific serotypes--Dengue 1, for example-- appeared to have a greater suppressive effect. No difference was observed in cell death or

metabolism. In conclusion, mixed infections of prototype dengue strains have different kinetics of replication as compared to single infections in cell culture. Strategies to develop live attenuated vaccines should investigate the kinetics of mixed infection during evaluation of candidate strains.

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MUNICIPALITIES IN PUERTO RICO WITH HISTORY OF HIGH INCIDENCE RATES OF DENGUE

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Dengue is a mosquito-borne viral infection that is endemic throughout the tropics worldwide. The incidence of dengue varies by season and may vary by city or region within a country. Information on historical trends of incidence can assist countries in identifying sites for vaccine trials and large cohort studies. We used data from the Puerto Rico Department of Health and the Centers for Disease Control and Prevention's (CDC) passive dengue surveillance system to determine annual rate of suspected and laboratory-positive dengue from 1990-2008 for the 78 municipalities. We calculated five-year average rates by municipality; 1990-1994 (outbreak period); 1995-1999 (outbreak period); 2000-2004 (non-outbreak period), and 2004-2008 (outbreak period). Municipality-specific rates were ranked for each time period, and those municipalities with the top twenty highest rates in each period were identified. There was a strong correlation between suspected and laboratory-positive dengue rates with sites having high levels of reporting also had high levels of laboratory-positive dengue. Only 2 municipalities had laboratory-positive dengue rates ranked in the top ten in all 4 time periods; 7 municipalities ranked in 3 of 4 time periods, and 12 municipalities in 2 of 4 time periods. From these municipalities only 11 municipalities with the highest historical incidence rates were clustered together in 3 regions; the central northern (Ciales, Florida, Manatí and Vega Baja); southeastern (Arroyo, Guayama and Patillas), and west central region (Adjuntas, Lares, Las Marías and San Sebastián). The combined populations by US Census 2000 of these regions were 139,516; 83,570 and 108,823, respectively. In conclusion, our data suggest that there are three regions with historically high rates of dengue that also have adequate sample size for large studies. Further research will focus on regional differences in population demographics, housing, climate, and environment that may put these communities at risk.

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DENGUE INFECTION AMONG SCHOOL-AGED CHILDREN AND ADOLESCENTS IN PATILLAS, PUERTO RICO: RESULTS OF A PROSPECTIVE SEROTYPE-SPECIFIC INCIDENCE STUDY, 2007

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Dengue is a mosquito-borne febrile illness caused by one of four serotypes of dengue virus (DENV-1 through DENV-4). Dengue is responsible for up to 500,000 annual cases of dengue hemorrhagic fever and 25,000 deaths globally. In Puerto Rico, endemic dengue is a major source of morbidity. Dengue surveillance in the municipality of Patillas reveals the highest rates among 10 to 19 year olds (13.4 laboratory-positives per 1,000 population in 2005). A prospective cohort study was conducted to describe dengue serotype patterns in children and adolescents. A random sample of 10 through 18 year olds attending schools in Patillas was selected. Between March and April 2007, 345 healthy students were enrolled, and had serum samples collected. A second sample was collected 12 months later. All samples were tested for IgM, IgG and neutralization antibodies using a MAC-ELISA, IgG-ELISA and microneutralization (MN) ELISA, respectively. Enrollment and 12-month prevalence, seroconversion, and DENV serotype were determined. The mean age of participants was 13.3 years and 45.5% were male. Only 3.5% reported prior dengue illness and none reported history of other flavivirus diagnoses or yellow fever vaccination. Of the 342 students with complete testing, 42.1% showed evidence of prior infection; all four serotypes were detected (DENV-1=7.0%, DENV-2=14.5%, DENV-3=28.2%, DENV-4=8.5%). Of the 308 with paired samples, 18 students (5.8%) seroconverted after 12 months; of which 5.6% had a primary DENV-3 infection, 77.8% had a secondary infection, and 1.7% resulted in indeterminate immune status. After 12-months, eight students had MN titer increases solely in the preexisting predominant serotype; none reported non-dengue flavivirus diagnoses, two traveled off the island (mid-west United States and US Virgin Islands), and none received a flavivirus vaccine during the study. In conclusion, almost half of the children and adolescents under 19 years of age in Patillas have been exposed to dengue. All four dengue serotypes have circulated through this group. Much higher incidence was found in this group than previously reported.

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EARLY IMMUNOLOGICAL CHANGES IN DENGUE VIRUS INFECTED RHESUS MACAQUES

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Dengue virus infection is increasingly becoming a worldwide major public health issue and is one of the most important mosquito borne human diseases in terms of morbidity and mortality. Wide spectrum of dengue diseases are known, ranging from asymptomatic, febrile-like illness, to potential life-threatening dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) characterized by thrombocytopenia and increased vascular permeability. Dengue is a timing illness, the appearance of clinical manifestations and the periods at the time of specimen sampling can vary among affected individuals. Most of the specimens that have so far been studied are obtained during or at the appearance of clinical signs of dengue. Thus, a comprehensive picture of events that precede the acute infection period is lacking. Previously we demonstrated that dengue hemorrhagic manifestations could be produced in rhesus macaque monkeys which were intravenously infected with Vero grown dengue virus serotype 2 at the dose of 1X107 PFU/animal. Peripheral whole blood collected prior to infection and every other day after the virus inoculation for two weeks was subjected to complete blood count, blood chemistry, and immunological FACS profiling. Compiling results from 7 experimental monkeys were following. Hemoglobulin, hematocrit, WBC, RBC, and platelets were consistently reduced at early days of infection. Fluctuations of lymphocytes and monocytes with modest reduction of neutrophils were seen. Distinct features of lymphocyte subpopulations, such as transient increase of CD3+ T cells, in particular the sub-phenotype of CD4+ T cells, transient reduction of CD20+ B cells and modest increase of NK cells, were observed. Platelet-leukocyte aggregation in M shape pattern was seen. Interestingly, comprehensive FACS analysis revealed that two striking subpopulations of CD45+ cells with distinct patterns were conspicuous; a sudden burst on day 1 and abrupt reduction of CD4+CD8+ cells on day 2 and remained at the same level thereafter, and a gradual reduction of sub-phenotype of CD41+CD61+CD62P- cells during the first few days after the infection. These comprehensive analysis and observations are impossible being documented in natural infection. Perhaps these immunological parameters may be a crucial factor, which may dictate in understanding the complicated pathogenesis of DHF/DSS.

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DENGUE ECONOMIC BURDEN IN THE AMERICAS: ESTIMATES FROM DENGUE ILLNESS

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Despite the growing burden of dengue, the global economic impact of this disease remains poorly documented. Information on dengue costs is now available for several countries but, to our knowledge, no estimate of the total costs induced by dengue worldwide or for endemic regions has been published to date. The objective of this study was to estimate the economic burden induced by dengue cases in the Americas using available information on costs and reported cases. Reported costs for 5 American countries (Brazil, El Salvador, Guatemala, Panama, Venezuela) (as reported previously) were used to estimate dengue costs in other American countries after adjusting for 1) differences in purchasing power parity with World Bank data, 2) differences in medical costs using the WHO-CHOICE database 3) differences in income using GDP per capita provided by the World Bank. Results are expressed in 2008 US dollars. Information on the number of dengue and dengue hemorrhagic fever cases and related deaths were those reported by PAHO over the 2000-2007 period corrected for under-reporting (using a factor of 6 for the base case, 1 to 10 in sensitivity analysis). The annual economic burden induced by dengue in the Americas was estimated to exceed $1 billion on average for the 2000-2007 period. Variations in incidence led to ranges in economic burden from $0.5 billion in a low incidence year (2004) to $2.1 billion in a high incidence year (2007). This economic burden is unevenly distributed among the 5 sub-regions identified by PAHO: 65% for the Southern cone (mainly because of Brazil), 16% in the Caribbean region, 10% in the Andean region, 9% in Central America and Mexico and less than 1% in North America. In conclusion, compared to previous country specific analyses, this study provides information on dengue costs for the entire American continent. Since the costs of vector control activities as well as the possible consequences of large outbreaks on the economic activity were not factored in, these results may still underestimate the overall economic consequences of dengue.

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DENGUE MENINGOENCEPHALITIS IN INDIA AND BANGLADESH

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Dengue virus(DENV) is the cause of one of the most important arthropod-borne diseases worldwide. There are an estimated 100 million dengue fever cases and 250,000 deaths due to DENV infections per year. Outcomes of dengue infection include asymptomatic infection, dengue fever (DF), and dengue hemorrhagic fever (DHF). Despite a few reports of DENV isolation from or genomic RNA detection in cerebrospinal fluid (CSF) from some encephalitis cases, meningoencephalitis clinical syndrome has not been widely attributed to DENV infection, particularly in areas where multiple flaviviruses co-circulate and one, such as Japanese Encephalitis Virus (JEV), is well-known to be associated with neuroinvasive disease. In collaboration with WHO, Centers for Disease Control and Prevention, and the Ministries of Health of India and Bangladesh, laboratory-based syndromic surveillance was undertaken for Acute Encephalitis and Meningitis Syndrome (AMES) at five sites in India and three sites in Bangladesh from March 2007 to September 2008. The AMES case definition is a person of any age, at any time of year with the acute onset of fever and either a change in mental status or new onset of seizures. From these cases, serologically confirmed Dengue associated cases accounted for 6.3% (N=121) of samples tested. To further investigate DENV as an etiology of meningoencephalitis in this study, CSF from AMES patients with Dengue IgM positive or equivocal results and with sufficient remaining volume were tested further by serotype-specific DENV real-time RT-PCR and nested RT-PCR, followed by genomic sequencing, and virus isolation. Of the samples tested (N=41), 68% (N=28) were DEN positive by at least one assay and 48% (N=17) were positive by more than one of the assays used. A ~500 bp region of the DENV genome coding for the nonstructural 5 protein was sequenced from 6 of the samples, 92% of the DENV RNA positives were identified as Dengue DENV-1, and 1 was identified as DENV-4. In conclusion, DENV infection should be considered as an etiology of meningoencephalitis in India and Bangladesh.

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EFFECTS OF INTEGRATION OF NEGLECTED TROPICAL DISEASE INTERVENTIONS ON VOLUNTEER HEALTH WORKER WORK-TIME IN NIGERIA

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The Carter Center, in conjunction with the Nigerian Ministry of Health and with support from the Bill and Melinda Gates Foundation, has expanded health services and integrated six neglected tropical diseases endemic to Nigeria. The primary mechanism for delivery of new and integrated packages is the community health worker (CHW) who, as a result of these expanded services, is distributing more drugs and products than before. Community leaders and CHWs are key actors in the integration strategy yet limited research has been done to show how the integration of NTD interventions impacts the workload of CHWs. The objective of this study was to quantify the CHW workload across varying intervention packages and to identify changes in workload associated with integration. The study team developed survey for CHWs and then interviewed 55 CHWs in 16 communities across 15 local government areas in Plateau and Nassarawa states. Survey data were analyzed using EpiInfo. Nearly sixty four percent (63.7%) of CHWs were involved in expanded and integrated interventions and 77.1% of that group reported an increase in workload ranging from 1 to 30 days extra work. There are a multitude of factors that influence amount of time spent on CHW work besides the distribution itself. The increase, decrease or lack of change in work-time caused by expanded or integrated interventions relies heavily upon the size of the coverage area, community cooperation, level of sensitization needed by the community towards the new drugs, the number of VHWs in the community and drug availability.

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PERCEPTIONS ABOUT HEALTH PROCESSES AMONG COMMUNITY MEMBERS FROM SMALL RIVERINE POPULATIONS IN THE PERUVIAN RAINFOREST

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Little is known about the processes that community members in the tropical rainforest of Peru follow to address day-to-day health issues. Therefore, the objective of this study was to describe the processes that

the inhabitants of Loreto, Peru utilize to address the health problems of young children and adults. This study was conducted in four small- to medium-sized communities along the Marañon and Huallaga rivers in the Peruvian Amazon. Focus groups were held with 34 community members aged 18 years or older recruited using purposive sampling. Participants documented the processes that community members follow to address important health problems faced by children under five years of age, adult males and adult females. Data were analyzed by synthesizing and comparing the flow diagrams produced by participants. For cases of pediatric diarrhea and pneumonia, the majority (75%) of participants wait three to six days prior to taking their child to a health facility. Home treatment provided during the wait consists of primarily natural remedies (65%) and some self-prescribed medication (35%) prior to formal medical treatment. Adult males typically wait one to two days prior to seeking professional care for stomach pain and approximately 80% of males never seek skilled care for presumed malaria. Most adult women (65%) with a urinary tract infection or vaginal discharge use natural remedies for one to four days before going to a health establishment. One-third of women wait one day before seeking professional care for vaginal bleeding. Natural remedies for different populations and health problems included drinks with herbs, fruit peels and bird beaks; baths with herbs, flowers and barks; and cigarette smoke. It is important to note that natural remedies often included medication acquired from a pharmacy. In conclusion, residents of these Peruvian Amazon communities utilize multiple methods to address health problems at home, based on their own diagnoses of their health issues and perceptions of which treatments are most appropriate. Further research needs to be performed to determine the best methods to optimize the utilization of appropriate health care and prevention services.

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PREVALENCE OF INTESTINAL PARASITES, ANEMIA AND MALARIA IN SMALL RIVERINE POPULATIONS IN THE PERUVIAN RAINFOREST

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People living in the tropical rainforest of Peru are at high risk of acquiring infectious diseases. It has been previously estimated that 60-100% of the population has intestinal parasites and that the prevalence of anemia is 44% in children under 5 and 33% in adult women. This study, conducted in rural Loreto, Peru, aimed to determine the prevalence of anemia and malaria in the population and intestinal parasitosis in young children. We randomly selected children under 5, their mothers and 15-49 year old individuals from four small riverine communities as part of a community-based health survey. Participating children provided a stool sample. We analyzed the samples for evidence of parasitic infection using direct light microscopy. Participants, except children <1 year, provided a blood sample. We measured the hemoglobin (Hb) level using the Hemocue® system and diagnosed *Plasmodium* infection using direct microscopy on thick and thin blood smears with Giemsa staining. We evaluated 157 stool samples and found an intestinal parasitic infection prevalence of 65%. The most common parasites identified were: *Ascaris lumbricoides* (43%), *Blastocystis hominis* (30%), *Entamoeba coli* (29%), *Trichuris trichiura* (26%) and *Giardia lamblia* (19%). We evaluated 1,002 participants, including 264 children under 5, for anemia and malaria. The prevalence of anemia was highest among children (37.5%), followed by their mothers (28.5%) and 15-49 year olds (12.2%). *Plasmodium vivax* was identified in a single participant, resulting in a prevalence of 0.1% in the sample group. In conclusion, the high prevalence of anemia in this population and intestinal parasitosis among children are similar to previous reports and suggest continued deficiencies in nutrition, hygiene, and public health infrastructure. Further information on nutritional patterns, quality of health services, and access to potable water and sanitation should be sought to identify potential areas of intervention.

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THE EFFECT OF WILDLIFE TRANSMISSION ON RABIES VACCINATION THRESHOLDS IN TANZANIA

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Vaccination of domestic dogs is the primary form of rabies prevention in the developing world. However, the circulation of the virus through co-existing wildlife populations may have an effect on the vaccination threshold required to eliminate the disease. We have developed a model that uses type reproductive numbers instead of the traditional R0 to determine the vaccination threshold required for interruption of disease transmission in settings where domestic and wildlife populations coexist. We have applied our model to contact tracing data from the Serengeti and Ngorongoro districts in Tanzania. Our model demonstrates that the circulation of rabies virus through the wildlife population does raise the level of vaccination coverage required to eliminate rabies infection from domestic dogs, but not so substantially as to be prohibitive to control efforts.

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STRENGTHENING MANAGEMENT CAPACITY OF PERSONNEL INVOLVED IN INTEGRATED INTERVENTIONS FOR NEGLECTED TROPICAL DISEASE (NTD) TO SUPPORT A MORE EFFECTIVE END EFFICIENT DELIVERY OF INTEGRATED INTERVENTIONS IN THE NIGERIA

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Most control and treatment programs for neglected tropical diseases (NTDs) focus on a single disease. Integrating these interventions could increase their effectiveness and reduce costs, easing the strain on public health systems. In addition to the technical challenges of integrating the different NTD protocols, public health staff face significant managerial challenges, including supervision, data management, and procurement logistics for drugs and intervention supplies. Integration also faces a natural resistance to a change of doing business, sharing resources, and assuming new responsibilities. Strengthening the management skills of NTD control personnel empowers them to address these challenges within the context of an active service delivery program. In Nigeria, The Carter Center assists the ministries of health of two states (Plateau and Nasarwa) in integrated disease control activities for onchocerciasis, schistosomiasis, lymphatic filariasis, trachoma, malaria, and Vitamin A supplementation. In collaboration with the Centers for Disease Control and Prevention (CDC) and Emory University, The Carter Center established the Sustainable Management Training Center (SMTC) to combine organizational change management strategy with a hands-on learning approach. Trainees apply newly learned skills to solve real management problems at their workplace while implementing the integrated interventions. Since July 2007, the SMTC has trained 118 personnel involved in integrated interventions in Plateau and Nasarawa state. Currently 30 applied management improvement projects are underway to improve the effectiveness and efficiency of the integrated interventions by using available local human capacity and institutional resources. One management improvement project resulted in an increase of the percentage of health facilities

reporting data on NTD cases from 11% to 82%. The SMTC program shows that management strengthening improves integrated interventions and could benefit other countries as they adopt similar approaches to NTD control.

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FEMALE GENITAL MUTILATION, ATTITUDE AND PRACTICES - A CASE STUDY IN RURAL GHANA

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The practice of Female Genital Mutilation (FGM) has attracted a great deal of condemnation worldwide. The practice is often carried out on woman and might be influenced by various cultural and religious beliefs. A cross-sectional survey in the Sissala district of the Upper West region of Ghana was carried out to evaluate the views of men and women, regarding the practice. A total of 197 respondents, 21% male, were selected through both systematic and simple random sampling and a structured interview guide was administered in 2000. Clinical examination of victims of FGM (n=30) was also carried out to identify the type(s) of FGM, defined as clitoridectomy or type I (surgical removal of the clitoris), excision or type II (surgical removal of the clitoris and part of the labia minor) and infibulation or type III (removal of the clitoris, labia minor and major). Of the 136 women interviewed, 84% had undergone FGM. Type I 17(56%) and type II 12 (41%) were the most prevalent. Most women (86%) had the operation done at infancy. Naming ceremony (62%) and rite of passage (9%) were carried out on the same day respectively. FGM as an Islamic requirement was the most predominantly cited reason by both victims (33%) and men (38%). Other reasons such as; chastity, fidelity in marriage, enhancement of matrimonial opportunities, promotion of female hygiene, and peer pressure were also cited as reasons for the practice. Knowledge of complications of the practice was generally very poor. Only 30% of victims and 47% of men were aware of any complications. In conclusion, the prevalence of FGM in some parts of Northern Ghana is still high. Contrary to earlier findings, this study has identified that the targeted age group for FGM in the Sissala district are infants. FGM as an Islamic requirement, carried out on the same day with naming ceremony, might also explain the trend towards infants.

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TOWARDS INTEGRATION OF NTD PROGRAMS IN TANZANIA: COORDINATION, COLLABORATION OR CONSOLIDATION?

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Five diseases have been targeted for preventive chemotherapy by WHO. These are Onchocerciasis, Lymphatic Filariaisis, Schistosomiasis, Soil Transmitted Helminthiasis and Trachoma. In Tanzania single disease programmes to control and in some instances to eliminate these diseases were established between mainly between 1998- 2004. Most of the programmes were linked to global single disease partnerships with little or no linkage to other similar programs in country and weak linkages to the health system. Taking into consideration the global focus on an integrated approach to control of these diseases, and building on the successes of the single disease programs, Tanzania is implementing integrated NTD control. The overall aim is to maximize the use of the minimum resources that exist whilst increasing efficiency and ensuring timely delivery of these effective interventions. The approach has been to use Community Drug Distributors (CDD's), Community own Resource Persons (CORPS) and in some instances schoolteachers to distribute the drugs for the five diseases at different time intervals This paper will describe the processes leading to integration in Tanzania and will mainly focus on integration in the activity and organizational domains. The paper will describe the development of an integrated strategic plan, governance, joint training programs and a common Monitoring and Evaluation Strategy. It will also discuss where integration is occurring (national, district and village) and how it is occurring. This programme initially began in two regions of Tanga and Morogoro and has expanded to three additional regions covering a total population of 9 million people. The achievements and challenges will be discussed at the various levels in these regions. The challenges include coordination of activities at the various levels and the complications linked to moving away from a single disease approach. The opportunities created by the integration process will also be addressed mainly in the context of strengthening a sub-district workforce that is capable of delivering effective interventions. The successes of this "rapid impact package" in the five regions of Tanzania will also be discussed.

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UNDER-UTILIZATION OF HEALTH CARE SERVICES FOR INFECTIOUS DISEASE SYNDROMES IN RURAL AZERBAIJAN

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Information on utilization of health services during an infection is essential for development of effective medical intervention strategies, including targeted education programs and public health efforts. Data on the prevalence of infectious syndromes provides information not otherwise available in populations with low health care utilization. A two-stage, probability-proportional to size sampling design was used to select 40 villages in northern Azerbaijan with populations <500 people. After providing informed consent, volunteers responded to a questionnaire recording demographics, health care utilization, and history of clinical syndromes. In the last 5 years, 6/768 (0.8%) volunteers reported being hospitalized for an infection, 6/768 (0.8%) visited a physician, 27/768 (3.5%) stayed in bed, and 144/768 (18.8%) took antibiotics without consulting a physician. In contrast, 338 illness episodes leading to antibiotic use were reported in a 5 year period with substantial regional variation observed. Only 4.5% of volunteers reported having any chronic medical conditions. Severe illness in the last 5 years included fever lasting more than 1 week (7), seizure (24), paralysis (3), and coma (3). Sixty seven (9%) reported illness in the last 2 weeks, consisting primarily of headache (28%), fatigue (15%), and joint pain (12%). Death of a family member in the last 5 years was reported for 78 households, with cardiovascular disease (21) and unknown (14) as the predominant causes. In conclusion, we observed a remarkably low utilization of health services, despite reported symptoms that would merit use. Widespread availability of antibiotics may deter health care use, and may contribute to the development of antibiotic resistance in this population. Research into the underlying causes of these health seeking behaviors should be conducted with the goal of improving health care delivery.

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SOCIAL CHANGE, ASTHMA AND ALLERGY IN LATIN AMERICA (SCAALA): PRELIMINARY FINDINGS

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Asthma is a chronic inflammatory disease of the airways. Although there is clear genetic susceptibility, the asthma epidemic in recent decades has been explained by social and environmental changes. IgE-mediated asthma is recognized as extremely important in developed countries, but there is growing awareness of the importance of non-atopic asthma. The findings from SCAALA (Social Change Asthma and Allergy in Latin America), a multidisciplinary research network based in Brazil and Ecuador investigating the social, environmental, and genetic determinants of asthma, broadly support this - in population-based studies among children in urban Brazil (Salvador) the proportion of wheezy children with positive allergen skin tests (SPT+) is only 34.4%, not much higher than the proportion of SPT+ in non-wheezers (26.8%), giving a population attributable fraction (PAF) of 26.5%. For rural Ecuador the corresponding numbers are 12.0% and 10.2%, respectively (PAF= 2.2%). Further, risk factors associated with atopic and non-atopic asthma in both populations were distinct. The findings have important implications for asthma prevention and control in Latin American populations.

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COST EFFICIENCIES OF NTD INTEGRATION

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Integration of interventions for NTDs is ongoing in two states in central Nigeria. One key programmatic objective is to assess whether cost efficiencies are gained as a result of integration. We assess the direct costs of integration in the health system using data collected over 3 years (2006-8), both retrospectively and concurrently. Health personnel at MOH state and LGA levels and NGO personnel involved in NTD implementation keep a daily work log of NTD activities to ensure that real time data on the key input of personnel time and effort are captured. Over 100 health personnel have completed monthly work logs that reflect changes over time from a single focus NTD to integrated NTDs. The activities have not substantively changed but efficiency has been gained in terms of time allocation of personnel, per diems and transport expense. This has resulted in an overall cost savings of $X from the baseline year 2006. The cost per NTD treatment varies from $X to $X across the LGAs as a result of different NTD packages and different roll out modalities. Inputs at the community level have also been monitored to assess the impact of integration.

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DETERMINANTS OF UTILIZATION OF OUTPATIENT HEALTH CARE IN GHANA

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Ghana is a low-income country with an estimated population of 24 million. Its health care system is a combination of public and private facilities as well as traditional healers. It remains unclear what factors influence utilization of health care in developing countries. Health care utilization, formal or informal, public or private, may be determined by several factors, including socioeconomic and demographic factors. Differences in perceptions regarding etiology of disease and confidence in health care providers may also determine the utilization of health care. Using multiple logistic regression, the probability of outpatient health care utilization by an individual, in event of illness, was computed as a function of age, ethnicity, gender, marital status, employment, geographic location, income, education and religion. Data for this analysis are the Ghana living standards survey round four (GLSS4, 1999) data, obtained from the Ghana Statistical Service. Overall, 25694 eligible household members were covered by the surveys and included for analysis. Utilizing health care was defined as consulting either a trained health care provider, traditional birth attendant (TBA), or a traditional and/or spiritual healer in the event of an illness. Per capta household expenditure was used as a proxy for income. Data was analyzed using SPSS statistics package (SPSS Inc, version 14, Chicago, IL) The results indicate that age, being married, divorce, male gender, basic education and income had a statistically significant impact on the utilization of health care. ($P < 0.05$). Basic compared to secondary and tertiary education, significantly increased an individual's log odds of seeking care ($P < 0.05$). A progressively higher income level, was significantly associated with an increased log odds of seeking and utilizing health care ($p<0.05$). In conclusion, the trend towards utilization of care by people with basic education could be explained by the broad definition of utilizing care, which includes the consultation of unconventional providers which are often not patronized by the highly educated. Affordability of health care, by virtue of a high income level, spousal influence and the vulnerability of the elderly to illness might explain the significant impact of these variables on health care utilization.

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APPLICATION OF HIGH DENSITY RESEQUENCING MICROARRAY RPM-TEI V. 1.0 FOR ANALYSIS OF SOIL AND DUST SAMPLES FROM THE MIDDLE EAST

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US personnel deployed to the Middle East as well as local population are subjected to high levels of airborne desert dust containing a significant fraction of particles in the inhalable size range. The dust is capable of carrying a range of toxic chemicals and diverse microbial organisms associated with their surfaces. Clinical syndromes associated with exposure to the desert dust that were not directly attributable to physical action of sand were reported but not much is known of their possible infectious origin. In the presented study the composition of the microbial communities associated with this dust was investigated by molecular methods on samples of surface sand or airborne dust from 15 sites in Kuwait and 4 sites in Iraq. Total nucleic acids were extracted from the samples and length heterogeneity PCR (LH-PCR) was employed to determine bacterial or fungal species richness and diversity. The nucleic acid preparations were also studied using high density resequencing microarray for detection of tropical and emerging infections (RPM-TEI v. 1.0), which was designed to detect a wide range of biothreat agents including most category A, B and C agents according to Center for Disease Control classification. LH- PCR indicated that samples harbor a variety of fungal and bacterial organisms. The sequences detected using RPM-TEI microarray included sequences from mycobacterium belonging to a class of rapidly growing mycobateria. Other organisms detected included bacillus, brucella, clostridium and Coxiella burnetti. Many of those pathogens are associated with infections or respiratory illness and some of them (bacillus, coxiella) are known for their resistance to drying and ability to be spread over long distances. The presence of infectious agents in the inhalable desert dust may pose a health hazard warranting further investigation to determine their impact on human health. Better understanding of microorganisms present in environmental samples is also necessary for efficient detection of biothreat agents.

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VIRUS ECOLOGY AND GLOBAL HEALTH: THE SHOPE LEGACY

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In this talk, I will discuss the Shope legacy in the arena of virus ecology and global health. My grandfather, Dr. Richard Shope was an internationally renowned virologist and the person who first isolated the influenza virus in swine in 1931, observing the ecosystem of Iowa pig farms. My uncle, Dr. Robert Shope was the world's most distinguished arbovirologist, known for his skillful detective work tracking down viruses in tropical ecosystems. Both were pioneers in using ecological approaches in their epidemiological studies, with implications for global health. In November 2003, University of Texas Medical Branch (UTMB) celebrated the completion of a biosafety level 4 laboratory, the first in the U.S. at an academic institution_the laboratory was named in Bob's honor ("The Robert E. Shope, M.D. Laboratory"). UTMB also established in his honor a Fellowship in Emerging Viral Diseases Research ("The Robert E. Shope, M.D. Memorial Fellowship"). Bob Shope was one of the early voices alerting policy-makers to the global health concerns of emerging infectious diseases. My name is Dr. Richard Shope III. I was born into the Shope family of virologists and had the opportunity to be immersed into science early on. I am a Science Educator with the Urban Ecology Working Group at Loyola Marymount University, helping inform teachers and students about urban ecology and the global health implications of climate change as it impacts urban regions, through a nationwide project, the Urban Science Corps, in which science-savvy undergraduates work as science coaches in urban communities. With the recent H1N1 outbreak and the movement of tropical disease into previously temperate climates, this presentation highlights the historical relevance of the Shope legacy, and current highlights of research conducted by recipients of the Robert E. Shope International Fellowship. I will also discuss the success of the Urban Science Corps, and its potential as a vehicle of global health education.

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THE ROLE OF COMMUNICATION SCHOLARSHIP IN THE PREVENTION AND CONTROL OF DISEASES OF GLOBAL IMPORT

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Communication is inherent in all aspects of tropical public health and medicine. However, communication scholarship and practice remain largely misunderstood, underfunded and underutilized, as acknowledged in the WHO Bulletin special edition on health communication (August 2009). Health communication theory and practice are essential to efficient and effective planning and implementation of public health programs. Endeavors such as: Conducting formative research to learn how health and disease are understood in rural communities in the developing world; attempting to alter individual and/or community attitudes and behaviors to improve public health; preparing messages for public media, including times of epidemics and/or disasters; and advising policy makers charged with local, regional and national public health policy all require practical and focused expertise that emerges from communication scholarship. Communication scholarship can help provide answers to questions such as: How can communication scholarship and practice enhance the planning, implementation and evaluation of interventions for improvement of tropical public health? What are the most effective mechanisms to facilitate collaboration and cooperation among professionals in communication and tropical public health? How can strategic communication promote the health of vulnerable populations? What is the role of narrative in health and healing? How is communication an agent for social change? Communication scholars share their perspectives on these and related issues where research, clinical practice, global public health and communication intersect.

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SPATIAL HETEROGENEITY OF SOIL-TRANSMITTED HELMINTHS: IMPLICATIONS FOR RAPID ASSESSMENT AND CONTROL

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Recent years have seen a renewed interest in the study and control of Neglected Tropical Diseases, such as soil-transmitted helminths hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*. A vital prerequisite to the implementation of drug campaigns to reduce the morbidity associated with this group of parasites is a sound understanding of geographic distributions of infection. In resource poor settings this requires rapid, cost effective survey techniques to identify populations at risk, which currently do not exist for soil-transmitted helminths. An important part of developing rapid survey techniques is the consideration of levels of spatial autocorrelation of the parasites concerned, as increasing levels of spatial autocorrelation lead to decreased precision of mean estimates. Semivariogram analysis revealed that hookworm displays spatial autocorrelation over large scales, and *A. lumbricoides* and *T. trichiura* display either spatial autocorrelation over small scales, or are randomly distributed. Using computer simulations of STH infection at public primary schools in Kenya, the effect of these varying levels of spatial autocorrelation on the performance of different sampling strategies will be investigated and results discussed.

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EFFICACY OF MASS DRUG ADMINISTRATION (MDA) OF ALBENDAZOLE IN THE REDUCTION OF SOIL TRANSMITTED HELMINTH INFECTION IN SOUTH INDIA: COMPARISON OF DATA FROM TWO ADJACENT DISTRICTS OF TAMIL NADU

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Soil transmitted helminth (STH) infections are a major public health concern, in tropical and subtropical countries. WHO has recommended periodic mass drug administration (MDA) with albendazole targeting primarily school children for control of STH. In India, the overall prevalence rates range from 12.5-66% with varying rates for individual parasites. Although the efficacy of antihelmintics has been well documented, the efficacy of mass drug administration (MDA) is yet to be established under field conditions. The state of Tamil Nadu in south India has introduced MDA for the control of STH in certain districts since 2001. The prevalence of STH among school going children in a district with MDA (Vellore) was compared with another district without MDA (Tiruvanamalai). A total of 19 schools (11 in urban Vellore, 4 in rural Vellore and 6 in rural Tiruvanamalai) were selected and stool samples obtained from children aged 6-14 years who attended these schools and whose parents provided informed consent. Out of a total of 1549 stool samples screened for the presence of STH infection using direct microscopy followed by McMaster technique, 76 (4.90%) were positive for at least one STH. Prevalence of STH in different schools range from 0-18.5%.The prevalence of STH in Vellore district was 4.5% (95%CI=2.5%-6.5%), whereas in Tiruvanamalai it was 5.7% (95%CI=1.7%-9.8%). Children attending the schools of urban Vellore had a higher prevalence of STH than their rural counterparts (5.7% vs. 2.6%, p=0.17). Similarly, children in rural Tiruvanamalai had

a higher prevalence of STH than rural Vellore (5.7% vs. 2.6%, p=0.22). Hookworm (2.3%) was the commonest helminth detected, followed by *Ascaris* (2%) and *Trichuris trichuira* (1%). *Ascaris* and *Trichuris* were more prevalent in urban children (p=0.003) whereas hookworm was more common in rural schools (p=0.002). Preliminary data suggests MDA to be effective in the control of STH infection under field conditions. Rural-urban differences in prevalence of STH in Vellore could be due to withdrawal of MDA program for past one year. Overall prevalence of STH in both districts seems to have declined over years.

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GENDER, AGE AND SOIL-TRANSMITTED HELMINTH INFECTIONS IN EARLY CHILDHOOD

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Gender and age considerations are important as large-scale deworming programs are planned to prevent and control soil-transmitted helminth (STH) infections (ie. *Ascaris*, *Trichuris* and hookworm infections). These considerations take into account peak prevalence and intensity of infections (eg. programs targeting school-aged children) and potential health impact (eg. antenatal care programs targeting pregnant women). In addition, a comprehensive gender and age lifecourse approach may provide further insight into the development and long-term effects of STH-attributable disability and disease risk. The objective of this study was to investigate gender and age differences in STH occurrence during childhood. Data were obtained from a comprehensive baseline nutrition and parasitological survey of 7-9 (n=168) and 12-14 (n=190) month-old children living in and around Iquitos in the Peruvian Amazon. Stool specimens were examined using the Kato-Katz method. All three STH infections begin to be acquired by 7-9 months of age (1.8%, 1.2%, 1.2%, respectively), with *Trichuris* infections progressing to 20.3%, and *Ascaris* infections to 11.8%, by 14 months of age. Hookworm infection did not increase between these timepoints. Boys and girls differed in terms of proportions infected with *Ascaris* and *Trichuris* at both age intervals, with girls having statistically significantly more *Trichuris* infections than boys by 12-14 months of age (p =0.039). Furthermore, of the 7 children with moderate and heavy infections (mostly *Ascaris*), six were in the 12-14 month age group and six were girls. These data, and data from other studies, illustrate differential profiles of gender and age in STH occurrence over the human lifespan. By mapping out STH prevalence and intensity over the lifespan, new insight may be obtained into the underlying determinants of infection.

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ANTHELMINTHIC USE IN THE FIRST TRIMESTER: LIMITATIONS OF CURRENT EVIDENCE AND IMPLICATIONS FOR LARGE SCALE DEWORMING PROGRAMS

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Large-scale deworming programs are advocated as an effective and efficient way of reducing the burden of disease due to soil-transmitted infections in high risk groups. One such high risk group is pregnant women, mainly because of hookworm-attributable anemia. Alleviating anemia during pregnancy has been shown to improve both maternal and infant health. However, there is concern about the inadvertent administration of anthelmintics to adolescent and adult pregnant women in their first trimester. This study was undertaken to review the evidence on inadvertent exposure to anthelmintics in the first trimester. Evidence on anthelmintic use during the first trimester of pregnancy was obtained from an extensive search of the peer-reviewed scientific literature. A total of 8 papers were identified. Two were single case reports; one summarized notifications to a pharmaceutical company; and 5 were epidemiological studies (3 cross-sectional and 2 case-control). The outcome of the two case reports (one with mebendazole use and the other with albendazole) were two healthy babies. The outcomes of half of the notices (25/49) to the pharmaceutical company were unknown. All of the epidemiological studies, conducted between 1996 and 2005, in different countries, contained methodological flaws, seriously limiting the interpretation of the data and, consequently, the conclusions drawn. These limitations include: incomplete data, inadequate inclusion/exclusion criteria, no statistical adjustment for important confounders, inappropriate comparison groups, unknown denominators, and incomplete reporting. Deworming programs targeting adolescent and adult women of reproductive age risk inadvertent exposure to anthelmintic drugs in the first trimester when pregnancy status may be unknown. Cumulative evidence to date is uninformative. Research is urgently needed to address this issue to ensure that deworming programs aimed at women of reproductive age are safe and beneficial.

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REDUCTION IN HOOKWORM INTENSITY OF INFECTION FOLLOWING TREATMENT WITH PRAZIQUANTEL IN LEYTE, THE PHILIPPINES

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This study was undertaken to assess the effectiveness of praziquantel in reducing the prevalence and intensity of hookworm infection among children and young adults in Leyte, The Philippines. Only one prior study conducted among children has examined the effect of praziquantel for hookworm infection, finding treatment reduced prevalence and intensity. Additional investigations in the context of different helminth co-infections, across varying ecological settings, and in broader age ranges are needed. We enrolled 626 males and non-pregnant females between 7-30 years of age who were infected with *Schistosoma japonicum* and otherwise healthy as part of an immunoepidemiologic study of *S. japonicum*. At enrollment, three stool samples were collected and each examined in duplicate by the Kato-Katz method to quantify the intensity of infection with *S. japonicum,* hookworm, Ascaris and Trichuris. All subjects were treated with a one-time split dose (60mg/kg) of Praziquantel. Stools were re-examined by the same methods every three months for 18 months following treatment. Paired t-test analysis showed a significant difference in hookworm eggs per gram (epg) at all time periods post-treatment when compared to baseline. The prevalence of hookworm infection declined from 61.0% at baseline to 46.2% at 3 months, and stayed relatively constant until 12 months, after which the prevalence went up to 54.6%. Three months post-treatment, there was a 41.5% decrease in the geometric mean eggs per gram (epg) among individuals infected at baseline. No significant declines in the geometric mean epg were observed for either Ascaris (0.17%) or Trichuris (0.11%) from baseline to three months. In conclusion, this study corroborates an earlier study conducted in an S. *mansoni* endemic area of Africa that found praziquantel reduced both the prevalence and intensity of infection with hookworm. We found similar efficacy in a broader age range with *S. japonicum* co-infection, suggesting that in areas where both are endemic, praziquantel may further reduce morbidity, particularly related to anemia.

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CATHEPSIN B- AND L-LIKE CYSTEINE PROTEASE ACTIVITIES DURING THE *IN VITRO* DEVELOPMENT OF *HYSTEROTHYLACIUM ADUNCUM* (NEMATODA: ANISAKIDAE), A WORLDWIDE FISH PARASITE

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Proteinases play an important role during the life-cycle of the parasites, and in the pathogen-host relationship as virulence factors. *Hysterothylacium aduncum* is a worldwide fish parasite nematode which has been related to non-invasive anisakidosis and to allergic responses to the fish consumption in humans. Cysteine proteinases have been related to the allergy to plant pollens, detergents and dust mites. Here, we study the occurrence of two families of cysteine proteinases (cathepsin B and cathepsin L) along the *in vitro* development of *H. aduncum* in a modified RPMI-1640 medium that enables development of the third larval stage (L3) into the mature adult. The L3s of this anisakid, isolated from fish *Trachurus trachurus* (intermediate/paratenic host), were used to start the culture. Samples for enzyme determination were as follow: L3 from fish, L3 after 48 h of cultivation, L4 after 14 days of cultivation, immature adult after 21 days of cultivation, and mature adult after 42 days of cultivation. Specific fluorescent substrates were used to determine the cathepsin activities (Z-FR-AMC for cathepsin B/L and Z-RR-AMC for cathepsin B). The detected activity with substrate Z-FR-AMC was identified as cathepsin L, with an optimum pH 5.5 (range 3.5-6.5). Cathepsin B activity was only identified with Z-RR-AMC, with an optimum pH 7.0-7.5 (range 5.0-8.0). The cultivation of the parasite led to an increased activity of both cathepsins (1.8-fold for cathepsin B and 6.3-fold for cathepsin L). These activities varied according to developing of the worm in culture time. So, the cathepsin L activity remained almost constant during ontogenic stages, at the same time as the cathepsin L one decreased after last molting (M4). According these activity variations and the optimum pHs, we suggest the cathepsin L is related to digestive processes while the cathepsin B could be involved in the cuticle remodelation, among other possible functions.

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INTENSITY AND PREVALENCE OF SOIL TRANSMITTED HELMINTHS IN HAITIAN COMMUNITIES

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Soil transmitted helminth (STH) infections are a major health problem in developing countries, including in Haiti. In the context of lymphatic filariasis elimination programs, mass drug administration (MDA) with albendazole (ALB), a benzimidazole (BZ) drug, and diethylcarbamazine (DEC) is administered annually. In addition to reducing microfilaremia, treatment reduces the intensity and prevalence of intestinal worms. The degree of selection pressure for drug resistance imposed by MDA has not been adequately monitored. A survey of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms was conducted, in one urban and four rural Haitian communities of the West department where MDA had not been conducted previously. Six months before the first MDA, stool examinations (n= 448) of these naïve communities were carried out by quantitative (McMaster) and concentration (sugar flotation) techniques. Prevalences of 5.8%, 12.8% and 13.6% were found for Ascaris, Trichuris and hookworms, respectively. When all available people from each community were re-examined 1 week before the MDA, the prevalence of STHs remained high. Post-MDA sampling is being conducted in the same communities to evaluate the drug efficacy. Moreover, molecular analysis will be done on STH eggs and larvae to assess the frequency of the genotypes associated with benzimidazole resistance.

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ACCIDENTAL FINDING OF A LIVE ANISAKID NEMATODE (*ASCARIDIDA: HETEROCHEILIDAE*) IN FRESH MARKET COD FISH FROM AN ATLANTA GROCERY STORE AND ITS IMPLICATION IN PUBLIC HEALTH

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Although there are a wide range of fish-borne parasitic diseases, only a few are of public health importance to humans. Surveys to ascertain the presence of fish parasites with a public health impact have not been performed in the US during the last two decades. Although recent estimates have not been made on the prevalence of anisakiasis in humans, approximately 50 cases have been reported in the US in the literature before 1988. Nevertheless, it is possible that the number of cases may be higher since anisakiasis can easily be misdiagnosed as presumed appendicitis. We report an incidental finding of live anisakid worms in a cod fish fillet obtained from an Atlanta grocery store. A semi-coiled worm-like object was spotted on a fresh cod fish fillet by a customer who brought the object for analysis. A close examination of the fish revealed six live worms. Morphologic examination confirmed the presence of three lips on the anterior end, a small mucron at the tip of the tail, and an anteriorly-directed intestinal cecum. This was sufficient to make a presumptive identification as genus *Pseudoterranova*. DNA was extracted from a small fragment cut from one of the worms; PCR amplification using generic 18SrRNA primers was performed to amplify a fragment of 800 bp. This amplicon was sequenced on both strands and this partial analysis of the 18SrRNA gene revealed a sequence that was identical to 2 Gen Bank entries, i.e., U94380 and U81575 from *P. decipieins* and *Anisakis* sp., respectively. Full length analysis of the 18SrRNA gene will be needed to finalize the characterization. The prevalence of anisakid parasites in fresh fish is unknown; surveys to assess this would be of public health interest. This finding highlights the need to adequately cook fresh fish before consumption.

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HUMAN CHRONIC INFECTIONS WITH *ASCARIS LUMBRICOIDES* AFFECT CYTOKINE PRODUCTION AND GENE EXPRESSION

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Chronic infections with *Ascaris lumbricoides* are associated with suppression of anti-parasite responses, but the mechanisms are poorly understood. We investigated mononuclear cell cytokine responses and gene expression/microRNA profiles in peripheral blood. Serial stool samples were collected from school children in rural Esmeraldas Province in Ecuador and examined for geohelminth infections and levels of anti-A. lumbricoides IgG antibodies. Children were stratified into 3 A. lumbricoides infection groups each of 20 individuals - no infection, low-level/intermittent infections, and chronic infections. PBMCs were stimulated with *A.lumbricoides* antigen and cytokine protein levels measured. RNA was analysed using an Illumina genomewide and microRNA arrays. Chronic ascariasis was associated with elevated levels of Th2 cytokines and IL-10, down-regulation of genes such as IL-8, and differential expression of microRNAs. The data indicate that chronic

ascariasis was associated with a modified Th2-like response (i.e. IL-5+/IL-10+). The data from gene expression and microRNA arrays provide novel insights into the genes and immunological pathways affected by chronic ascariasis.

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A NOVEL AND POWERFUL CLASS OF NEMATICIDES FOR SOIL-TRANSMITTED NEMATODE INFECTIONS

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Soil-transmitted nematodes, hookworm, *Ascaris*, and *Trichuris (*HAT*)*, are amongst the most prevalent human parasites, infecting more than 1 billion of the poorest peoples around the world. We have limited number drugs for treating these parasites, and, for practical reasons, only one (albendazole) is commonly used in mass drug administration (MDA). All our current drugs have limitations as used in MDAs, including moderate efficacy against hookworms, low efficacy against *Trichuris*, and threat of drug resistance. Therefore there is great need for new human anthelmintics and anthelmintic combination therapies. We are developing Crystal (Cry) proteins from the soil bacterium *Bacillus thuringiensis* as new anthelmintics. This bacterium has evolved a large family of proteins that are designed specifically to target and kill invertebrates while being safe to vertebrates. We have found that some of these proteins are able to cure helminth infections in mice model and hamster model of human helminth diseases. Here we will discuss our latest work on the development of these proteins, including efforts to develop Cry proteins as effective single dose anthelmintics and as combination anthelmintic therapies with nicotinic acetylcholine receptor agonists such as tribendimidine. In particular, we will highlight how we can use the free-living nematode *C. elegans* to develop more potent Cry protein variants and study anthelmintic combination interactions. We will then discuss how this data translates into efficacy studies in hleminth infections in mice.

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CHARACTERIZATION OF THE IMMUNE RESPONSE TO THE LARVAL PHASE OF *ASCARIS SUUM* INFECTION AND IDENTIFICATION OF *ASCARIS* ANTIGENS EXPRESSED DURING THAT PHASE

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Ascariasis is the most frequent soil-transmitted helminth. *Ascaris lumbricoides* infection occurs in greater than 25% of worldwide population with an estimated total of 1.2 -1.4 billion infected people; it is immunologically comparable to *A. suum* porcine infection and in some regions, the two can co-exist. *A. suum* infection, similar to *A. lumbricoides*, results from fecal-oral transmission with ingestion of infective eggs that hatch in the intestine; Larvae released in the intestinal lumen access the venous portal circulation where they mature. Final larval maturation occurs with migration to the lungs, at day 7 of infection, where larvae penetrate alveoli and small airways inducing an inflammatory response including production of IgE, mast cell degranulation, histamine release and eosinophilia. Larvae are swallowed and mature into the adult form in the small intestine, where females lay infective eggs. This project consists of immunoscreening an *Ascaris suum* lamba phage gt11 larval cDNA library obtained from the USDA with serum from previously infected animals challenged with re-infection. Samples of serum from day 7 after inoculation will be used to identify the most frequent immunogenic *A. suum* antigens during the larval phase of the disease. The most frequent antigens will be useful in development of better diagnostic assays and in the understanding of *A. lumbricoides* infection. Presently, diagnosis of human ascariasis is made from microscopic detection of eggs in stool, which occurs when the parasite has matured into an adult form in the intestinal lumen. This method therefore targets the chronically infected population but does not permit diagnosis in the early stages of the infection when larval migration causes significant asthma-like pulmonary pathology, most frequently in children. The significance of this study lies in its enormous potential in the understanding of immune response to *A. lumbricoides* infection, particularly to the larval stage responsible for the pulmonary manifestations, with the ultimate goal of developing improved diagnostic tools.

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EFFICACY OF SECOND-LINE THERAPY (PROTEASE INHIBITOR BASE-REGIME) AFTER NON NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NNRTI) BASED HIV TREATMENT FAILURE

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Failure of first-line therapy (NNRTI) is unavoidable in a proportion of HIV infected patients. According to WHO guidelines, protease inhibitor (PI) base- regime is widely used as second-line therapies. In source-limited countries are problematic, because the expense of PI. This study aimed to assess the efficacy of the second-line therapies according to single/double PI base-regime. Additionally, whether the use of boosted indinavir shows a difference in the efficacy. This descriptive, observational retrospective study was retrieved medical records during 2004-2006 at Bamrasnaradura Infectious Diseases Institute, Thailand. Single and double PI base-regimes were compared according to CD4+count, plasma viral load (pVL), bodyweight, and adverse events. If boosted indinavir is enclosed, were reviewed. Of 86 patients were included, 66.3% were male, 64 of 84 (76.2%) were classified as CDC category C3. Of 52 (60.5%) patients recieved GPO-vir as first-line regime. The median time from detection of treatment failure to start of second-line therapy was 39 (0-237) weeks, the median CD4+count and pVL at start of second-line therapy were 13 (0-872) cells/mm^3 and 98,900 (141-750,000) RNA copies/mL, repectively. The initial bodyweight was 54 (31-83) kg. At 24$\pm$12 and 48 $\pm$ 4 weeks, the increasing of CD4+ count, median pVL, bodyweight and adverse events showed no significantly different between single and double PI base-regimes, regardless of indinavir used. Eight cases had a treatment failure. In conclusion, distributions of complete pVL suppression increaseing CD 4+ count and bodyweight were similar in single and double PI base-regimes at 48 $\pm$ 4 weeks. Boosted indinavir as the most used PI in this patient group, did not show a significantly different to other protease inhibitors. This study gives the importance knowledge of selected second-line regime. Especially, the resourse-limited country.

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EFFECT OF HIV- 1 SUBTYPES A AND D ON DISEASE PROGRESSION IN HIV INFECTED PATIENTS WITH SEPSIS IN UGANDA

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Whether the different subtypes of HIV-1 affects HIV disease progression is not well known. While various studies have shown no association, most recently, some studies in Brazil as well as Uganda have shown that HIV disease progression can be affected by the subtype. In Uganda, subtype D has been attributed to faster HIV disease progression compared

to subtype A, with a mortality hazard ratio of 1.26. Given the high prevalence of sepsis in HIV infected patients and the high mortality in these patients attributed to sepsis, we set out to determine if severity of sepsis or mortality due to sepsis would be greater in patients infected with HIV-1 subtype D compared to subtype A. A prospective cohort study was done enrolling HIV infected patients (including both incident and prevalent cases) who presented with sepsis. Participants were enrolled from a larger cohort of patients with sepsis who presented to Mulago and Masaka Hospitals in Uganda from 2008 to 2009. HIV-1 subtyping was done by sequencing of the gag and env gene, followed by phylogenetic analysis. Information collected included; participants' demographics, CD4 count, antiretroviral drug use, severity of sepsis, WHO clinical stage and opportunistic infections (prior and present). Outcomes of interest were in-hospital and 28 day mortality. Of the 41 samples subtyped, 25 were subtype A (71.4%) and 10 were subtype D (28.6%). Both groups were similar in age and gender distribution, CD4 count (<50), ARV use, and WHO clinical stage. In the subtype A group, 8 patients (32%) died in the hospital, compared to subtype D with 3 (30%) deaths. Neither the mortality rate nor the time to death was statistically different. Length of stay in the hospital was not statistically different between the two subtypes either. In conclusion, so far, no difference is seen between the two subtypes in terms of disease progression, however given the small sample size, further studies of larger samples would be needed.

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HIV DISEASE AND BLOODSTREAM INFECTIONS AMONG FEBRILE PEDIATRIC HOSPITAL ADMISSIONS IN NORTHERN TANZANIA

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Few studies describe the contribution of pediatric HIV and of HIV co-infections to hospital admissions in sub-Saharan Africa. We prospectively studied febrile pediatric admissions to one hospital using modern laboratory methods. We enrolled consecutive admitted patients aged ≥2 months and <13 years with current or recent fever during one year in Moshi, Tanzania. A standardized clinical history and physical examination were done and hospital outcome recorded. HIV antibody testing, standard aerobic blood cultures and malaria film were done. Early infant HIV diagnosis by HIV-1 RNA PCR was done for those aged ≤18 months. HIV-infected patients also received serum cryptococcal antigen testing and CD4-positive T-lymphocyte percent (CD4%). Of 480 patients enrolled, the median age was 43 months (range 5 months-13 years), 205 (42.7%) were female, and 68 (14.2%) were HIV-infected. Provisional clinical diagnosis of HIV disease was made in 50 (10.4%) and of malaria in 282 (58.8%). Of HIV-infected patients the median (range) CD4% was 17 (0, 56)% and 2 (2.9%) had positive serum cryptococcal antigen tests. Of 447 (93.1%) with blood cultures, 18 (4.0%) grew pathogens. Of blood cultures with pathogens, 6 (33.3%) were *Streptococcus pneumoniae*; 5 (2.8%) *Salmonella* Typhi; 3 (16.6%) *Escherichia coli*; and 4 (22.2%) grew other pathogens. *Plasmodium falciparum* was identified on blood film of 7 (1.7%). HIV infection was associated with *S. pneumoniae* (odds ratio 30.0, p<0.001) bloodstream infection (BSI), but not with *S.* Typhi, *E.coli,* or *P. falciparum* BSI. Inpatient death occurred in 36 (7.5%) patients. In conclusion, *S. pneumoniae* is a leading cause of bloodstream infection among pediatric admissions in Tanzania and is closely associated with HIV infection. Malaria is over-diagnosed clinically, whereas the prevalence of HIV is underestimated. HIV and HIV co-infections contribute to a substantial proportion of pediatric febrile admissions underscoring the value of routine HIV testing.

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THE EFFECT OF AIDS BEHAVIORAL INTERVENTION ON CHINESE OVERSEAS WORKERS

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The objective of this study was understand the knowledge, attitude, behavior and concept mastering of AIDS that the Chinese overseas workers have. Explore effective methods of AIDS related behavioral intervention, to better prevent AIDS. We independently designed a survey form, adopting the anonymous questionnaire (KABP) method. We had 1855 Chinese overseas workers who came to our center for health check do the survey twice before and after AIDS behavioral intervention. The overseas workers had certain understandings to AIDS, but they only had a vague knowledge of AIDS high-risk behavior, and didn't know much about prevention. The majority of overseas workers were willing to receive interventional education. In conclusion, there are risky factors which could lead to AIDS spread among overseas workers. It's imperative to do behavioral intervention on them.

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MOLECULAR CHARACTERIZATION OF HIV-1 IN NICARAGUA

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The first case of HIV/AIDS in Nicaragua was not discovered until 1987, and to date there is no published data regarding the distribution of HIV genotypes in Nicaragua. In 2007, a collaborative project was established to evaluate the genetic distribution of HIV-1 in Nicaragua. We analyzed a total of 106 strains from HIV-seropositive samples and classified the strains genetically. Of these strains 93 (88%) grouped within subtype B in the *gag* gene and 74 (70%) grouped within subtype B in the *env* gene. The remaining gene sequences could not be amplified initially and remain to be classified. Genetic variability in a subtype may introduce diversified motifs, which may serve as markers of the geographic origin of certain HIV-1 strains and their Circulating Recombinant Forms. Therefore we further analyzed the V3 loop of the *env* gene product to identify specific motifs that may be indicative of genetic heterogeneity among the subtype B strains. The prevalent tetrameric amino acid sequences within the V3 loop were found to be GPGR (51%), followed by GPGK (13.4%), GPGS (7.5%) and HGPGR (1.3%). In summary, through molecular genotyping we documented the predominance of subtype B among HIV-positive Nicaraguan patients, although a subset of the HIV-1 strains remain to be identified. Not all B subtype strains shared the same characteristics, which may be indicative of different origins among subtypes. Phylogenetic analyses will be useful to document the relationships between the Nicaragua subtypes and other Central America subtypes. Further studies are necessary to determine the extent of genetic variability within other genomic regions of the HIV-1 strains.

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DECREASED ANTIVIRAL ANTIBODY RESPONSES IN SHIV1157IP-INFECTED RHESUS MACAQUES COINFECTED WITH *SCHISTOSOMA MANSONI*

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The effect of schistosome infections on host immune responses has been proposed to increase infection with HIV-1 and perhaps contribute to the apparent discrepancy of HIV/AIDS in sub-Saharan Africa compared

to areas where parasitic worms are not endemic. We have previously demonstrated that rhesus macaques with *Schistosoma mansoni* infections have increased susceptibility to SHIV infection and demonstrate increased viral replication compared to animals infected with SHIV alone. Other studies have demonstrated that schistosome infection alters host immune responses to bystander antigens, but this has not been demonstrated for host responses to immunodeficiency virus antigens in *S. mansoni*-infected monkeys. Here, we compared antibody responses to HIV-1 gp41 peptides in animals intravenously infected with SHIV1157ip after 15 weeks of *S. mansoni* infection to those of animals infected with SHIV1157ip alone. Using an ELISA with a standard curve to normalize between assays, we found that monkeys infected with schistosomes produced lower levels of IgG to gp41 after infection with virus than animals infected with SHIV1157ip alone (P = 0.005). These data suggest that persons infected with schistosomes may be less able to produce immune responses to viral antigens, which may in turn affect disease progression.

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PREVIOUS EXPOSURE WITH CEPHALOSPORINS AND MACROLIDES BUT NOT COTRIMOXAZOLE AS A RISK FACTOR FOR COLONIZATION WITH MRSA IN HIV-INFECTED CHILDREN

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MRSA infections in community accounted for 3-11% of *S. aureus* infections in resource-limited regions of Asia especially due to availability of over-the-counter antibiotics, their frequent self-administration for inappropriate indications and taken for irregular durations, low cost and substandard quality. We would like to expand those data by a small cohort of 102 HIV-infected children from Cambodia treated with HAART for 4 to 5 years. Of 102 HIV-infected children in the orphanage House of Family run by St. Elizabeth University, 62 were colonized within last 58 months with *S. aureus*, which caused 149 episodes of infection, 118 MRSA and 31 MSSA. Proportion of MRSA colonization was very high at the baseline (75-100%) without any previous known hospital contact as well as during first 24 months, then dropped to 57,1% and 43% after 27 respectively 30 months but then rose again to the level at baseline of 76% after 51 months. Meta-analysis which analyzed exposure of antibiotics as a risk factor for MRSA infection showed that risk of acquiring MRSA was increased by 1.8-fold in patients who had taken antibiotics. Quinolones increased risk 3-fold, glycopeptides 2.9-fold, cephalosporins 2.2-fold and other β-lactams 1.9-fold. When looking for previous exposure with antibiotics one month before infection, children infected with MRSA were significantly more frequently receiving oral 2nd and 3rd generation cephalosporins (67% vs. 23%, P<0.01) and macrolides (47% vs. 23%, P<0.05). However aminopenicillins, quinolones and cotrimoxazole were not significantly related with MRSA in HIV-infected children. Quinolones were less associated with MRSA in our group of children because of less frequent use in children. The prevalence of MRSA remained high but did not increase despite frequent antibiotic treatment of *S. aureus* infections during entire 58 months assessed period. Use of cephalosporins and macrolides were significantly related to the increased of MRSA infection but surprisingly despite children were receiving cotrimoxazole during the entire period of 4 to 5 years for prophylaxis as recommended by WHO, use of cotrimoxazole was not related to resistance selection. Emergence of MRSA in a resource-limited countries in Asia fuelled by uncontrolled antibiotic use outside hospital settings may pose a significant threat for risk group of patients such as our group of HIV-infected children from Cambodia.

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MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* ISOLATED FROM HIV-INFECTED PATIENTS IN MALAYSIA

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Cryptosporidium isolated from HIV-infected patients in Malaysia which were initially identified by using modified Ziehl-Neelsen staining technique were confirmed by nested PCR targeting a partial region of the SSU rRNA gene. PCR product were purified and sequenced in both directions and consensus sequences were aligned with sequences of *Cryptosporidium* SSU rRNA gene in the Genbank to determine the genotypes. The overall prevalence of *Cryptosporidium* based on microscopy and nested-PCR were both 18.6%. *C. parvum* were identified in 14 patients, *C. felis* and *C. hominis* were found on 1 patient respectively. The predominance of *C. parvum* suggests a higher probability of zoonotic transmission among the HIV-infected patients in Malaysia. Most of the patients had CD4 count lower than 50.

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EPIDEMIOLOGICAL INVESTIGATION OF ACUTE CHAGAS DISEASE IN THE PERUVIAN AMAZON

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In December 2007, a case of acute Chagas disease in an 8 year old girl well documented case of the disease in Loreto Department in the Peruvian Amazon indicated that Trypanasoma cruzi was endemic in the region. Further review demonstrated 9 reported cases since 2003 primarily in remote indigenous communities. In March 2008, study of family members of the acute case and a seroprevalence study in residents of the community of San Pedro de Shishita (45 households) located about 1 hour by fast boat from Pevas was carried out. *Trypanosoma cruzi* infection of the index case was confirmed by trypomastigote morphology in thick smears and blood culture of epimastigotes and observation of amastigotes in histological sections of heart muscle of Balb / c mice experimentally infected with blood from the index case. Among the 16 family members of the acute case, none were positive by thick smear (n=14) or xenodiagnosis (n=3). ELISA and indirect immunoflorescence (IIF) in 104 community members including family members, 1 individual (26 y/o female) had antibodies to *T. cruzi* (0.96%). Entomological collections were carried out in and around the household of the acute case; samples of Panstrongylus geniculatus and Rhodnius pictipes were collected in the bedroom of the acute case, in the peridomestic area of the house and in nearby households. None of the triatomid bugs collected were infected with T. cruzi during the investigation which occurred 3 months after the case was detected. In the same population 6 of 104 (5.76%) were positive for *Plasmodium vivax* and 3 (2.88%) for microfilariae. The case had no travel history outside her community indicates active transmission of *T. cruzi* within the jungle regions of Peru.

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CONCOMITANT-DISTANT LESIONS AS A POTENTIAL RISK FACTOR FOR ANTIMONY TREATMENT FAILURE IN ULCERATED CUTANEOUS LEISHMANIASIS IN PERU

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Increased rates for failure in leishmaniasis antimony treatment have been recently recognized worldwide. While several factors could influence treatment strategy and outcome, disease severity (associated with, but not restricted to, the size and the number of lesions) has a broad and notable

variability on the outcome of this treatment.To determine risk factors for antimony therapy failure in ulcerated cutaneous leishmaniasis patients, we assessed several "lesion severity" parameters in a randomized case-control study conducted in Peru. Among 87 antimony-treated patients, 18 cases (21%) failed the treatment, as judged by the persistence or worsening of existing lesions or the appearance of new lesions, while 69 cases (79%) were cured based on the complete scarring of lesions and the disappearance of inflammatory signs within 3 months following treatment completion. The measurements for severity included the size and number of lesions, and the occurrence of concomitant-distant lesion defined as the appearance of multiple lesions in different parts of the body (head, arms, trunks and legs) within a range of 15 days. The probability of treatment failure was modeled in a multiple logistic regression after adjusting for potential confounders including the disease time, the size of leishmanian skin test, as well as basic clinical, epidemiologic, and demographic parameters.The best multiple logistic regression model explained 49% of the variability of failure and remarkably showed a 23-fold increase in the risk of treatment failure in patients presenting concomitant-distant lesions compared to other patients (OR=22.8, 95% CI: 1.4~365.8). This high risk factor was only surpassed by the *L. (V) braziliensis* species as a main risk factor (OR=37.9, 95% CI: 3.0~484.8) (2). Another interesting finding was that the total area of lesions appeared to be a protective factor after normalization (OR=0.46, 95% IC 0.23~0.92). We found a positive correlation between the concomitant-distant lesions and treatment failure. Since monitoring the concomitant-distant lesions is relatively simple and non-invasive, it could serve as a potential indicator to predict treatment failure, after adjusting for patient age and occupation and parasite genotype.

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INSULIN-LIKE GROWTH FACTOR-1 ENHANCES THE INNATE IMMUNE RESPONSE OF MACROPHAGES FROM WELL-NOURISHED MICE BUT CAN NOT RECTIFY THE DEFECTIVE IMMUNE RESPONSE OF MACROPHAGES FROM MALNOURISHED MICE

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Malnutrition contributes to 55% of all childhood deaths from infectious diseases in developing countries. I have previously described a murine model of human weanling malnutrition in which malnutrition leads to: (1) increased visceralization after infection with Leishmania donovani; (2) defective macrophage pro-inflammatory cytokine response (decreased levels of TNF-α, IL-10, and NO and increased levels of IL-6 after IFN-γ/LPS stimulation); and (3) an increased ratio of immunosuppressive prostaglandins compared to pro-inflammatory leukotrienes (after LPS stimulation). Insulin-like growth factor 1 is a hormone produced primarily in the liver that reflects the anabolic/catabolic balance and that also affects immunocompetence and macrophage differentiation. In our model, serum levels of IGF-1 were reduced by 71% in the malnourished mice compared to the well-nourished mice. We hypothesized that IGF-1 primes macrophages for enhanced innate immune response and that in the malnourished mice lower levels of IGF-1 contribute to the defective macrophage pro-inflammatory response. In macrophages from well-nourished mice, pretreatment with IGF-1: (1) increased LPS-stimulated production of TNF-α by 100%; (2) decreased the prostaglandin/leukotriene ratio by 75% and IL-6 release by 66%; and (3) had no effect on NO or IL-10 levels. In IGF-1-primed/LPS-treated macrophages from the malnourished mice, there were: (1) trends toward decreased IL-6, TNF-α, and IL-10 release: (2) a small increase in NO (10%); and (3) no effect on the prostaglandin/leukotriene ratio. Thus, in macrophages from well-nourished mice, IGF-1 has significant stimulatory effects on the innate immune response. However, in macrophages from malnourished mice, supplemental IGF-1 is unable to restore the defective pro-inflammatory macrophage mediator response. The IGF-1 resistance of macrophages in this model of polynutrient malnutrition may be due to antagonism of IGF-1 action by excessive levels of IL-6 and corticosteroids observed in malnutrition or the absence of adequate zinc for IGF-1 signaling.

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A COHORT-BASED STUDY OF *LEISHMANIA MAJOR* INFECTION AND CUTANEOUS LEISHMANIASIS IN MALI

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Cutaneous leishmaniasis (CL) due to Leishmania major has been reported from Sahelian regions of Mali. The absence of current information on the status of CL in the country led us to conduct a cohort study in Kemena and Sougoula, two endemic villages in central Mali. In May 2006, we administered a leishmanin skin test (LST) intradermally to all permanent residents to determine baseline prevalence of *Leishmania major* exposure. LST negative individuals were re-tested 1 and 2 years later to estimate the annual incidence of *L. major* infection. From March 2007 to April 2008, we conducted bimonthly active case detection for CL to estimate disease burden. We also determined the seasonal variation and the prevalence of *L. major* infection for *Phlebotomus duboscqi* sand flies (main vector in the study area). The baseline prevalence of L. major exposure (i.e., LST+) in 2006 was 45% (301/663) in Kemena and 20% (173/867) in Sougoula. This prevalence increased significantly with age from 10% and 6% in children <5 years old to 75% and 40% in adults >40 years old in Kemena and Sougoula respectively. The first annual incidence rates of L. major exposure (i.e., conversion from LST- to LST+) were 19% (54/287) in Kemena and 6% (32/569) in Sougoula. The second annual incidence rates were similar: 17% (32/191) in Kemena and 5.7% (27/478) in Sougoula. Over 1 year of active case detection, only 4 LST+ individuals developed CL. Sand fly collections showed a relatively high density of *P. duboscqi* throughout the year and *L. major* was identified by PCR in 9.7% (161/1668) of *P. duboscqi* pools containing 1-20 sand flies each. Although these 2 villages are only 5 km apart, with a stable transmission of *L. major* over a 2-year period, they differed markedly in the prevalence and annual incidence of *L. major* exposure. We are presently investigating the underlying factors responsible for this difference in transmission intensity as well as the absence of clinically apparent skin lesions.

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TRYPANOSOMA EVANSI ANTIBODY LEVELS IN SOME EDIBLE FISHES IN KOLKATA, INDIA

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Recent studies in the city of Kolkata revealed that caprine animals are highly infected (45%) with *Trypanosoma evansi*. A serological survey also revealed that about 13% of human population was positive for *T. evansi* antibody. In the state of West Bengal in India, being one of the major states of the country thriving on fisheries which derives about 8000 tons per annum, a study on the *T. evansi* antibody levels in fishes appeared to be an important one to us. Nine different common edible fishes were selected for this study. 72 samples of blood of these fishes were collected from 10 different markets of Kolkata, West Bengal, and the antibody levels were measured by CATT/*T. evansi* kit obtained from

Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. This is a highly specific card agglutination test for trypanosomiasis that uses *Ro Tat* 1.2 - a predominant variable antigen type of *T. evansi*. *T. evansi* antibody was positive in 22 samples (30.5%), there was no species specificity and except two markets, positive samples were found in all other markets.Thus a significant number of edible fishes in Kolkata were found positive when tested for *T. evansi* antibody.

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NOVEL PRIMARY MEANS OF TRANSMISSION OF VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis is a fatal zoonotic disease usually associated with tropical areas. In many areas canines serve as the domestic reservoir host. The etiologic agent is an obligate intracellular protozoan, *Leishmania infantum*. In 1999, an outbreak of a canine visceral leishmaniasis was reported in a Foxhound kennel in New York, and since that report, several other outbreaks have occurred across the United States in additional Foxhound kennels. Our group has been following a cohort of over 500 of these dogs to better determine how this disease is transmitted among dogs in the United States and what factors may predispose to infection and disease. Based on several litters of pups, in one instance whelped in our bio-containment facility, there is strong evidence that transplacental vertical transmission occurs in these dogs, perhaps driving primary transmission within this population. Another possibility for transmission includes a more traditional vector-borne method, as a possible vector species, *Lu. shannoni,* does exist in many arboreal river valleys within the United States. Direct contact, through kennel fights and other blood to blood contact have also been suggested as a means of transmission. Transmission of disease in these dogs leads to a seropositive incidence approaching 9.8% and a quantitative PCR incidence of 22.8% in our cohort. Ongoing studies are providing evidence as to which dogs are more likely to be infected and what the risk of zoonotic transmission is in this country.

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SPATIO-TEMPORAL PERSPECTIVES ON PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN PERI-URBAN AREQUIPA, PERU

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Chagas disease, caused by *Trypanosoma cruzi* and transmitted by blood-feeding triatomine insects, infects an estimated 8 million people in Latin America. To improve understanding of *T. cruzi* infection in humans in peri-urban contexts, a cross-sectional seroprevalence study was conducted in the district of La Joya, 30 kilometers from the city of Arequipa. Of 1,333 study participants, 101 (7.6%) were confirmed positive for *T. cruzi* infection by ELISA and IFA. Prevalence of infection increased significantly by quartile of age: 0.3% (1-13 years), 4.7% (14-24 years), 9.3% (25-37 years), 16.9% (38-90 years); (χ2=73.1, df=3, p<0.001). Prevalence of infection also increased significantly by quartile of time of residence within the study area: 1.1% (1-5 years), 4.2% (6-11 years), 7.1% (12-21 years), 18.4% (22-72 years); (χ2=81.0, df=3, p<0.001). Kernel density estimate ratio analysis, evaluated with a 50-meter bandwidth, found no areas of substantially increased relative risk of *T. cruzi* infection that exceeded 95% tolerance limits. K-function difference analysis, evaluated at spatial scales from 10 to 500 meters, found minimal clustering of *T. cruzi* infection that exceeded 99% tolerance limits. Age-specific seroprevalence patterns suggest that *T. cruzi* transmission was interrupted in the study area in the recent past. The absence of areas of substantially elevated relative risk of *T. cruzi* infection, as well as the minimal degree and dispersed scale of clustering of infection, suggest that *T. cruzi* transmission within the study area has occurred over many years. Together, results of spatial and temporal analysis suggest that *T. cruzi* infection is endemic in the study area, and that both local transmission and migration may contribute to the prevalence of *T. cruzi* infection in the region.

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PREDICTIVE FACTORS FOR UNFAVORABLE OUTCOMES IN THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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We assessed treatment outcomes in patients with stage II human African trypanosomiasis (HAT) treated according to a traditional dosing schedule (TDS, 3 series of 3 intravenous (I.V.) injections, one injection per day with 3.6 mg/kg melarsoprol at 7 day-intervals, n = 95), or a short dosing schedule (SDS, 10 consecutive daily injections of 2.2 mg/kg melarsoprol, n = 160), or difluoromethylornithine (DFMO, I.V. infusion 400 mg/kg/day for 14 days, n = 37). Functional recovery was assessed in relation to the clinical status, dosing schedules, and biological changes in the cerebrospinal fluid (CSF). The predictive values of selected parameters were determined through logistic regression multivariate analysis using SPSS software. Unfavourable outcomes i.e. loss of autonomy and/or death (up to 8% and/or 24%, respectively, in the SDS-group) were closely associated to extant neurological impairment at admission ($P < 0.01$), simultaneous presence of parasites in blood/nymph node juice and CSF, and higher CSF protein content. Studies are needed to refine clinical protocols to help assessing the subject's clinical status prior to drug allocation in HAT, to clarify relationships between CSF modifications and clinical deterioration, and develop novel, safe, and affordable therapies.

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EXPANDING BELT OF HUMAN AFRICAN TRYPANOSOMIASIS (SLEEPING SICKNESS) IN PALLISA DISTRICT, UGANDA

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This abstract describes the recent Sleeping Sickness epidemic reported from Pallisa district of Uganda. Ever-since May 2008 when a six-month old baby, along with an elderly woman, reported to NaLIRRI Sleeping Sickness Hospital from Pallisa District, we set out to find the epicenter of the epidemic with a multi-disciplinary survey that included medical, veterinary and vector biologists to ascertain underlying epidemiological factors creating the outbreak. A visit was made to Budaka to the east and Namutumba to the west of Pallisa district and by July 2008 six more cases were detected in Nabiswa up to Tirinyi where the uncle to the baby died one month after the baby was diagnosed making us believe from

verbal interviews that he baby-sat the sick child regularly. By Nov 2008 we received an eight year old boy from Putiputi sub-county to the north-east of Kibuku and others from Namutumba across the Mpologoma River. In 2009 a one and half month old child reported and was later established to be related to the eighty year old man who had been admitted earlier to NaLIRRI hospital in 2008. Another multi-disciplinary survey in Tirinyi sub-county will be conducted in June around the home of the elderly man and baby discharged recently after treatment at NaLIRRI to document on the outbreak. Parameters underlying the epidemiology of Rhodesiense sleeping sickness in SE Uganda including environmental and landscape dimensions will be typed. Civic leaders and technocrats in Pallisa district as well as biologists and socio-economic scientists from NARO, the Ministry of Health, Animal Industry, Environment, Makerere University HAT community as well as the press will participate in a baraza to quantify the extent of the outbreak and report that a focus had spread to cover 100 square kilometers in one year a focus.

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CHARACTERIZATION OF UNKNOWN GPI ANCHORED PROTEINS IN *TRYPANOSOMA BRUCEI BRUCEI*

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An in-silico screen of the genome of the pathogen *Trypanosoma brucei* yielded several unknown hypothetical proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. Trypanosomes undergo several differentiation steps during development in the tsetse fly. Upon ingestion, bloodstream trypanosomes differentiate into the procyclic form and inhabit the fly midgut. Procyclic parasites migrate to the proventriculus and differentiate into epimastigote cells. Epimastogotes continue to the salivary glands where they attach and differentiate to mammalian infective metacyclic trypomastigotes. Finally, metacyclics develop into the bloodstream form in the mammalian host after inoculation by the fly during a bloodmeal. The genes carrying GPI anchor motifs were searched for signal peptides and other conserved domains. Homology with genes in related pathogens *Leishmania major* and *Trypanosoma cruzi* were noted. Of the twelve genes selected for further evaluation, 9/12 had known conserved domains and 9/12 were shared with *L. major* and *T. cruzi*. Gene specific primers were designed for each gene. To determine if these unknown genes were preferentially expressed in particular developmental stages, RNAs were prepared from parasite infected salivary glands, proventriculus, and midguts, as well as procyclic culture and bloodstream parasites. cDNAs were prepared and normalized by PCR using trypanosome α-*tubulin*. The normalized templates were tested with gene-specific primers for the twelve genes. No detectible product was obtained for 3/12 primer pairs. All other primer pairs yielded bands, and showed differential expression in different host tissues. Quantitative PCR will be used to determine the degree of up- or down-regulation. These data will provide further information on the role these genes may play in infection processes in the tsetse fly or vertebrate hosts, and increase our broader understanding of this complex interaction.

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MOLECULAR CHARACTERIZATION OF KINESIN^{CAAX} IN *TRYPANOSOMA BRUCEI*

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Trypanosomatids including *Leishmania* species, *Trypanosoma cruzi*, and *Trypanosoma brucei* contribute to the infectious disease burden of the world's most poor nations. Identifying essential proteins in these infectious kinetoplasts will lead to investigation of novel drug targets. These parasites rely heavily on kinesins and microtubules for motor functions as they lack intermediate filament based cytoskeleton components and most actin-based cell motors. One predicted kinesin, Kinesin^{CAAX}, conserved in *T. brucei*, *T. cruzi*, and *Leishmania major,* has the CAAX box motif for posttranslational addition of a farnesyl group. This lipid modification creates a hydrophobic tail that allows proteins to interact with the cell membrane, membrane-bound organelles or other cellular proteins. Studies in mammalian cells and yeast have shown that various lipid modifications are essential for activities in the cell such as proliferation and apoptosis. Our data show Kinesin^{CAAX} binds and moves along microtubules in the presence of ATP. Functional analysis in *T. brucei* by RNAi knockdown of Kinesin^{CAAX} reveals this protein is essential for parasite viability. Kinesin^{CAAX} knockdown parasites have an increase of G2 cells in the cell cycle and a deficit in normal chromosomal replication. Currently we are investigating the localization of Kinesin^{CAAX} in *T. brucei* bloodstream cell lines. We predict Kinesin^{CAAX} is a target of the *T. brucei* protein farnesyltransferase and this farnesyl group addition facilitates interactions with cellular cargo. Posttranslational modifications and farnesylation in particular are novel and little studied areas in the kinesin field and in parasite molecular biology. These studies have significant implications to aid development of therapeutics to combat these infectious parasites.

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LEISHMANIA INFANTUM ALDOSE REDUCTASE: EXPRESSION WITH MOLECULAR CHAPERONES, PURIFICATION AND KINETIC STUDIES

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Aldose reductase (EC 1.1.1.21), a NADPH-dependent oxidoreductase catalyzing the reaction of a broad range of aldehydes, has been implicated in the methylglyoxal catabolism (as reported previously), a toxic glycolysis byproduct. Enzyme activity assays in total protein extracts from *Leishmania infantum*, a trypanosomatid responsible for human leishmaniasis, revealed that this enzyme is active and is part of the methylglyoxal catabolic system. A candidate for *Li*AKR gene was identified by homology in the *L. infantum* genome, showing 40% and 51% identity with yeast and human aldose reductase genes, respectively. After unsuccessful attempts to obtain soluble *Li*AKR in *E. coli* expression systems, a yeast complementation strategy was adopted and enzyme activity was measured in protein extracts of a yeast null mutant for *gre3* gene (coding for aldose reductase) transformed with an expression plasmid containing the *L. infantum* gene. *K*m values for methylglyoxal and NADPH and the specific activity were determined by following NADPH oxidation in presence of methylglyoxal. Being similar to the ones in *L. infantum* extracts, *K*m values confirmed the enzyme's identity. Soluble protein was only obtained by over-expression in engineered *E. coli* strains containing a chaperone system, DnaK/DnaJ/GrpE, DnaK/DnaJ/GrpE/ClpB, GroESL or DnaK/DnaJ/GrpE/ClpB/GroESL, which were coordinately co-overproduced with the recombinant protein optimizing *de novo* folding. The purified *Li*AKR revealed to be stable and kinetically active. *L. infantum* aldose reductase was identified for the first time in this trypanosomatid genome. Its expression and purification optimization will be particularly useful for further biochemical and structure-function analysis. As far as drug targeting is concerned, studying this enzyme is of utmost importance, not only to understand the methylglyoxal metabolism in this infectious organism, but also to unearth crucial differences relatively to its human counterpart.

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EXPRESSION PROFILING USING *LEISHMANIA DONOVANI* GENOMIC MICROARRAY FOR THE IDENTIFICATION OF NOVEL VACCINE TARGETS FOR VISCERAL LEISHMANIASIS

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Leishmania is a protozoan parasite that causes human diseases widespread in tropical regions around the world, ranging from spontaneously healing skin lesions in cutaneous leishmanisis to life-threatening visceral disease. In India, *Leishmania donovani* is responsible for visceral leishmaniasis (VL) and Post kala-azar dermal leishmaniasis (PKDL) while *L. tropica* is responsible for cutaneous leishmaniasis (CL). During infection the extracellular insect forms (promastigotes) undergo rapid differentiation to intracellular amastigotes that proliferates in phagolysosomes of mammalian macrophages. To identify virulence-related genes in *L. donovani* we used microarray based expression profiling in Indian isolates at promastigote and amastigotes stages as well as in an intermediate stage during transformation. Transcript profiling led to the identification of 67(1.5%) differentially expressed clones in the intermediate stage and 226 (5.3%) in terminally differentiated amastigotes. Majority of the differentially expressed clones corresponded to genes involved in metabolism, cellular organization and biogenesis, transport and genes encoding unknown function. Microarray results were validated by Northern blots and RT-PCR. Among the genes upregulated at the amastigote stage, a trypanosomatid specific protein of 27 kDa (P27), Ubiquitin activating enzyme E1 (LdUba5), Parasite surface antigen 2 (PSA2) and MAP kinase (MAPK) were selected for further studies. Transcripts of all these 4 genes were demonstrated *in vivo* in VL bone marrow samples by Real Time PCR. Further we evaluated the transcript abundance of these genes in skin lesions of PKDL and CL patients with respect to *Leishmania* tubulin as internal control, taking two known amastigote specific genes, A2 and A1, as positive control. The results highlighted substantial differences in gene expression patterns *in vivo* in the three different forms of Leishmaniasis, indicating the involvement of selected genes in the disease pathogenesis. Functional characterization of such genes is underway to elucidate their role in virulence of the parasite and their potential as vaccine targets.

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AN UNUSUAL HSP70 PRESENT IN RARE *LEISHMANIA VIANNIA* ISOLATES FROM PERU

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Cusco is a hyper endemic area for Leishmaniasis in Peru. Most patients are infected with *Leishmania (Viannia) braziliensis,* although other species like *L. (V.) lainsoni* and *L. amazonensis* have been described in a considerable much lower proportion.Six isolates from Cusco, three of them obtained from naturally infected *Lutzomyia* sandflies and three from patients with cutaneous lesions, presented a very unusual *Hae III* PCR-RFLP pattern for hsp70 encoding gene. They were quite different from the canonical *L. braziliensis* pattern present in hundreds of isolates from different endemic areas. On the other hand, PCR-RFLP of the ribosomal internal transcribed spacer (ITS) confirmed that these parasites belonged to the *Viannia* and not to the *Leishmania* subgenus. Hsp70 DNA sequencing revealed the same sequence for both human and insect vector obtained isolates. It presented more than 40 nucleotide substitutions when compared with the hsp70 sequence of 15 *Leishmania (V.) braziliensis* isolated from Cusco and 5 *L (V.) peruviana* from the Andean highlands. The latter two species had a hsp70 sequence with a very high homology to the corresponding gene reported for the M2904 international reference strain. Thirty of the substitutions were non synonym codons representing twenty different aminoacid residues. The substitutions did not affect those aminoacids that are strictly conserved along evolution, from yeast to trypanosomes, except for a change of a glutamic acid in two different positions. Firstly, Glu 566 by Asp, which did not change the net charge of the protein. Second, Glu 582 by the polar non charged serine at the C-terminal Substrate Binding Domain. The latter change is a very interesting fact because it has been implied in the contact between the nucleotide binding domain and the α helical sub domain in yeast hsp70 dimer molecules. Parasite identification will establish if we are dealing with a rare hsp70 gene that replaced the canonical one of *Leishmania braziliensis* or that the six isolates correspond to a novel taxonomic *Viannia* subgroup.

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IDENTIFYING MICRORNAS THAT ALTER MACROPHAGE SUSCEPTIBILITY TO INFECTION BY *LEISHMANIA*

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Macrophages are a heterogeneous population of innate immune effector cells. Stimulation of macrophages with inflammatory cytokines and microbial products results in M1 macrophage polarization. Macrophages can also be activated by an array of stimuli that result in polarization distinct from the M1 macrophages (M2a, M2b, and M2c). M1 macrophages are associated with stronger microbicidal activity against intracellular pathogens, such as *Leishmania*, than those with M2 phenotypes. There are many differences in gene expression, cytokine secretion, and display of cell surface markers between the M1 and M2 subsets of macrophages. MicroRNAs (miRNA) are capable of repressing the expression of hundreds of proteins. We hypothesize that the dynamic changes seen in the polarized macrophages are controlled, at least in part, by the action of miRNAs. Recently, three miRNAs (miR-132, miR-146a, and miR-155) were reported to be up-regulated in macrophages stimulated by LPS, which activates macrophages toward M1-like phenotypes. Monocyte-derived macrophages (MDMs) were polarized via stimulation with IFNγ and LPS (M1), IL-4 (M2a), IgG and LPS (M2b), or TGF-β1 (M2c). RT-PCR revealed unique profiles of CXCL9, IL-10, CD206, and CCL18 transcripts were regulated by the distinct polarized MDM phenotypes. Bio-Plex cytokine assays detected IL-6, IL-1β, and TNFα secretion by M1 MDMs and to a lesser extent by M2b MDMs. Only M1 MDMs produced large quantities of IL-12(p70). These results confirm that our experimental stimulation conditions directed proper macrophage polarization. Surprisingly, MDMs do not induce miR-146a and miR-155 under M1 polarizing conditions or by LPS treatment alone. We have initiated experiments to identify miRNAs regulated in polarized MDMs. The functional relevance of each candidate miRNA will be tested by ectopic introduction of the miRNA into macrophages followed by testing changes in susceptibility to infection by *Leishmania*. Such observations could ultimately contribute to the development of novel small RNA-based forms of therapy.

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THE ANTIMICROBIAL PEPTIDES AARP-1 AND RP-1 DEMONSTRATE MICROBICIDAL ACTIVITY AGAINST BOTH *LEISHMANIA MAJOR* AND *LEISHMANIA CHAGASI INFANTUM*

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Antimicrobial peptides occur naturally and are considered essential to the innate immune system. Kinocidins, a unique group of microbicidal chemokines, function in the bloodstream and consist of a distinct extended N-terminal domain, a C-terminal α-helical domain, and a γ-core structural domains. Direct microbicidal activity is attributed to the C-terminal α-helix that interacts with microbes to alter membrane structure or inhibit macromolecule synthesis. RP-1 and variant AARP-1 are synthetic peptides designed to imitate structure-activity relationships

of certain kinocidin helices. RP-1 has efficacy against Gram-negative bacteria, Gram-positive bacteria and fungi. RP-1 retains efficacy in complex biomatricies such as human blood and blood fractions. Efficacy is greater in these environments than in artifcial media. *L. major* is a cause of cutaneous leishmaniasis; *L. chagasi infantum* is a cause of visceral leishmaniasis. *Leishmania* strains engineered to express firefly luciferase were directly exposed to varying concentrations of RP-1 and AARP-1 *in vitro*. Using bioluminescence as a measurement of parasite viability, we demonstrate that exposure to micromolar concentrations of AARP-1 and RP-1 results in complete killing of both species of *Leishmania* promastigotes *in vitro*. Additional work is required, but these results suggest that such peptides have potential to be used therapeutically against visceral and cutaneous leishmaniasis in humans.

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COMPARATIVE PROTEOMIC ANALYSIS OF IMMUNODOMINANT 30-34 KDA PROTEINS OF *TRYPANOSOMA CRUZI* BY DIFFERENT MASS SPECTROMETRY METHODS

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Trypanosoma cruzi is the etiological agent of Chagas' disease, an illness that widespread in Latin America, affecting 13 million of people. Transmission usually occurs by infected triatomine bugs, but in non-endemic areas, as U.S. and Europe, transmission by contaminated blood transfusion is the major route of infection.Detection of antibody to *T. cruzi* by serological techniques is the main choice for diagnosis of infection in the chronic phase of the disease. Crude extracts, purified and recombinant proteins, as well as synthetic peptides of the parasite are used as antigens. Differences in protein profiles obtained from different strains of the parasite and differences in the human humoral immune response can lead to variations in sensitivity and specificity rates in endemic and non-endemic areas in different parts of the world. Furthermore, the absence of a gold standard method generates the interest in searching for new antigens. In previous work, parasite proteins of 30 to 34 kDa have demonstrated high specificity for diagnosis of Chagas'disease when immunoblot analysis was used. Identification and characterization of such proteins are needed. Recently, proteomic approaches have been successfully used as tools for discovering new antigenic molecules of *T. cruzi* and other parasite agents. In the present study, a 30-34 kDa protein fraction was purified by preparative electrophoresis and initially analyzed by two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-TOF and MALDI-TOF/TOF). The results were compared with those obtained by LC-MS/MS (ESI-Q-TOF) analysis. The total comparative analysis showed 26 protein types with emphasis to heat shock protein 70, P28, ribosomal proteins and prostaglandin F2α synthase (PGF2 synthase) which was shown to be the predominant. The 2-DE/western blot analyses using monoclonal antibodies and sera samples from chagasic patients showed high percentage of reactivity with the PGF2 synthase, indicating that this could be a candidate to be used as antigen in the serodiagnosis of Chagas' disease.

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TRYPANOSOMA CRUZI -INDUCED INFLAMMATION IN ADIPOSE TISSUE

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Trypanosoma cruzi infection causes an intense inflammatory response. We reported that adipose tissue is an important target of *T. cruzi* infection in a mouse model. In this report we describe the response of adipose tissue to this infection through the synthesis and release of adipokines, cytokines and chemokines and their contribution to various signaling pathways and systemic inflammation during acute and chronic infection. CD1 mice were infected with the Brazil strain and brown adipose tissue (BAT) and white adipose tissue (WAT) were analyzed 15 and 100 days post infection by immunoblot and qPCR. Both types of adipose tissue displayed a massive infiltration of macrophages upon infection, a down-regulation of adiponectin (a fat-derived adipokine), a change in peroxisome proliferator-activated receptor-γ (PPARγ), an upregulation of the major three MAPKs (ERK 42/44, pP38, pJNK) and an increased expression of Toll-like receptors-2, 4 and 9. In addition, infection caused an upregulation of cytokines (TNF, IFNγ, Itgb2 and Ltb) and an increase in the expression of Ccl2, Ccl5, Ccl7, Ccl8, Ccl4, Ccl6, Cxcl9, Cxcl10 and Il-1a, Il-1b, Il-18 and Il-17. Interestingly, we found that different signaling pathways are involved in the regulation of immune response in infected BAT and WAT. In infected BAT, a down-regulation of PPARγ, activation of pJNK, induction of TNF and IFNγ as major pro-inflammatory markers and upregulation of additional cytokines and chemokines was observed. In contrast, WAT displayed an up-regulation of PPARγ, TLR-9 and ERK42/44 while no significant changes in mRNA expression levels were seen for TNF and IFNγ. In infected WAT, PPARγ antagonizes many effects of TNF. TNF secretion in infected BAT suppresses adiponectin, an anti inflammatory adipokine as well as PPARγ, resulting in an inflammatory phenotype of the adipocytes. Overall, these data implicate adipose tissue as an important target of *T. cruzi* infection contributing to the systemic inflammatory response seen in this infection.

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EVALUATION OF INHIBITORY EFFECT OF A *TRYPANOSOMA BRUCEI* CALCIUM CHANNEL ANTIBODY (ANTI-TBCC1) IN VITRO

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Trypanosoma brucei is a protozoan parasite that causes Human African Trypanosomiasis (HAT, sleeping sickness). 2-300 million people are exposed to this disease and an estimated 50,000 deaths occur annually in sub-Saharan Africa. Thus, new targets are sought for development of effective vaccines or drugs against infection. Existing drugs used to treat HAT are toxic and often times lethal and vaccines developed against HAT have been unsuccessful due to parasite evasion of the host's immune system by antigenic variation. An *in vitro* drug study using commercially available Ca^{2+} channel blockers effectively inhibited parasite proliferation and survival at micro Molar concentrations when compared to Pentamidine controls, indicating the presence and importance of L-type Ca^{2+} channel in survival. A putative L-type *T. brucei* Ca^{2+} channel (TBCC1) was identified and characterized and specific anti-TBCC1 antibodies were produced and used to locate the channel in pericellular, nuclear and flagellar pocket regions by immunocytochemistry. We hypothesized that treating parasites with anti-TBCC1 antibodies will interfere with [Ca^{2+}] homeostasis and proliferation in parasites. To test this hypothesis we performed antibody blocking assays testing different concentrations of anti-TBCC1 on blood stage cultures of *T. brucei in vitro*. The results indicate that anti-TBCC1 is immunogenic, binds pericellular and nuclear membrane, interferes with Ca^{2+} homeostasis, and inhibits *T. brucei* proliferation *in vitro*.

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DETECTION OF *TRYPANOSOMA CRUZI* ANTIGEN IN SKELETAL MUSCLE OF FETUSES WITH MORPHOLOGICAL STRUCTURAL ANOMALIES FROM MICE WITH ACUTE CHAGASIC INFECTION

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The congenital Chagas disease is a public health problem that allows the uncontrolled transmission of parasites from one generation to other. In this study the NMRI mice (Mus musculus) female were inoculated intraperitoneally with $22x10^3$ metacyclic trypomastigotes from the M/HOM/BRA/53/Y *Trypanosoma cruzi* strain. To obtain pregnancies, mice were mated with males at day 15 after infection. The results showed high levels of patent parasitemia in the mice with 30 days of infection and 20 days of pregnancy in comparison with infected unmated mice. The infected pregnancy mice were sacrificed at day 20 of gestation and the fetuses with their placentas were removed and evaluated. The infection by *T. cruzi* in pregnancy mice with highest parasitemia affect the intrauterine developed of some of their fetuses. Morphological and structural muscular-skeletal anomalies similar to protuberances were found in 3 (15%) fetuses from two mice infected. In one fetus the protuberances were seen on the body dorsal side and another on the left footpad base; another fetus developed his right footpad came out from right part of his face and another fetus developed a lump in the left leg above the subscapular region. Histological sections of placenta, heart and skeletal muscle of the fetuses staining with Hematoxilin and Eosin, showed inflammatory infiltrate with lymphocytes, macrophages and monocytes, and discrete myositis and myocarditis. The immunocytochemical assay using Peroxidase anti Peroxidase, showed *T. cruzi* antigen in the placenta and skeletal muscle of the fetuses with morphological alterations. The amplification of DNA of *T. cruzi* by Polymerase Chain Reaction (PCR) showed positive reaction in 18% of placental tissue from mice infected pregnant. The samples of heart and skeletal muscle of the fetuses from infected mice with *T. cruzi* did not show DNA of T. cruzi. The presence of *T. cruzi* antigen in the skeletal muscle of fetuses with morphological anomalies from two mice infected, might be related with the virulence, tropism and to be associated with the biological and genetic characteristic of *T. cruzi* strain used in experimental infection of female mice.

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CHARACTERIZATION AND EXPRESSION OF A NOVEL SECRETORY NUCLEASE, *LMEX*NUC^S, IN THE HUMAN PATHOGEN *LEISHMANIA MEXICANA*

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Leishmania spp infect over two million people each year worldwide, causing a range of symptoms from simple cutaneous to fatal visceral disease. *Leishmania* promastigotes reside and multiply extra-cellularly within the gut of their sand fly vector and as obligate intracellular amastigotes within the phago-lysosomal system of mammalian macrophages. All *Leishmania* are purine auxotrophs and thus must salvage these essential molecules from their host environments to survive. In that regard, here we identified, characterized and expressed a unique 35kDa secretory nuclease in *L.mexicana* (*Lmex*Nuc^S), which plays a role in purine salvage in this organism. Structural analyses showed that it contains all five conserved domains characteristic of members of the P1/S1 nuclease family. The *Lmex*Nuc^S is *N*-linked glycosylated and DTT-sensitive. In addition; our results demonstrated that the *Lmex*Nuc^S shares high levels of homology with several other trypanosomatid P1/S1-like nucleo-hydrolases. Results of Western Blot and molecular analyses showed that *Lmex*Nuc^S is constitutively secreted by both parasite developmental forms. Further, using a variety of molecular techniques we found that the *Lmex*Nuc^S enzyme is expressed at higher levels by amastigotes than promastigotes. Immunoprecipitation and enzyme activity assays showed that the *Lmex*Nuc^S expressed enzyme possessed nuclease activity against both single and double stranded DNA as well as various synthetic polynucleotide substrates. Cumulatively, our results indicate that the *Lmex*Nuc^S enzyme must play an essential role in purine salvage to facilitate both the growth and development of this human parasite.

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IMPACT OF COMBINED TREATMENT OF ALLOPURINOL AND BENZNIDAZOLE ON TOTAL AND *TRYPANOSOMA CRUZI*-SPECIFIC T CELLS IN HUMAN CHRONIC CHAGAS DISEASE

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Chagas disease affects 24 million people from Southern California to Central and South America. Etiological treatment in long term *T. cruzi* infection is controversial, despite the fact that BZ treatment showed significant protection from progression of heart pathology. We have previously found that a significant proportion of BZ-treated subjects exhibit an initial increase in IFN-γ producing T cells specific for *T. cruzi* between 2-6 months post-treatment followed by a decrease to undetectable levels 12-36 months after treatment in association with decreased levels of antibodies specific for *T. cruzi* recombinant proteins. An improvement in early differentiated antigen-experienced total $CD8^+$ T cells at 24 months post-treatment was also observed in these subjects. Even though the use of combined drugs has been proved to be effective in other chronic infections, assessment of combined treatment in human Chagas disease has not been performed. This study was aimed to evaluate the effect of sequential treatment with two drugs allopurinol (AL) and BZ on T cell responses in subjects chronically infected with *T. cruzi*. AL was administered for 3 months (600 mg/day) followed by 1 month of BZ (5 mg/kg/day) in 11 *T. cruzi*-infected subjects. The frequency of *T. cruzi*-specific IFN-γ-producing T cells significantly increased after AL treatment and decreased thereafter in a sustantial proportion of subjects evaluated. Total naïve ($CD45RA^+$ $CCR7^+$ $CD62L^+$) and central memory ($CD45RA^+$ $CCR7^+$ $CD62L^+$) $CD4^+$ and $CD8^+$ T cells significantly increased after AL along with the raise in *T. cruzi*-specific T cells and maintained at 24 months post-treatment. These findings support that treatment with AL and BZ during chronic Chagas disease has a substantial impact on parasite-specific immune responses and also induced an earlier effect on the total T cell compartment compared with treatment with BZ alone. It remains to be elucidated whether the improved in naïve and central memory T cells is due to a direct effect of AL on the host immune system.

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ANTIMALARIAL DRUG DISPENSING AMONG COMMUNITY DRUG PROVIDERS IN IBADAN NIGERIA: A CROSS-SECTIONAL SURVEY

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The drug of choice for the treatment of uncomplicated malaria in Nigeria was changed from chloroquine to artemisinin-based combination therapy (ACT) with a preference for artemether-lumefantrine in January 2005. Drug providers play an important role in provision of antimalarial drugs in the community. This study aims to evaluate the knowledge,

attitude and practice of community drug providers in the use of ACT in Ibadan southwest Nigeria. In a cross-sectional survey, using a 55-item self administered questionnaire, the demographic characteristics, antimalarial drug knowledge and dispensing practices of 100 community drug providers in an urban city in southwest Nigeria were assessed. Means (±SD) were determined; t-test and ANOVA were used for comparison of means and chi-square to test associations. P-values were statistically significant when ≤ 0.05. Of a hundred drug retail outlet operators interviewed 31 were trained pharmacists, 2 were nurses while another 21 had received tertiary education in various disciplines. Most (45%) antimalarial drug prescriptions were for artesunate (ART) while 65.9% of dispensers entertain ≥ 50 verbal requests for antimalarial drugs/ week. Artesunate was the most often dispensed antimalarial drug in filling prescription (48.8%) or recommended by the drug provider (34%) to clients who requested for no specific antimalarial drug. Others monotherapies frequently prescribed were sulfadoxine-pyrimethamine (13%), chloroquine (8%) and amodiaquine (7%) while arthemether-lumefantrine constituted 7%. 92% of respondents couldn't correctly mention any of the criteria for diagnosis of severe malaria. Most dispensers were unaware of the drugs of choice the treating acute uncomplicated or severe malaria as contained in the National Treatment Guidelines even though 51% of respondents claimed to be aware that there is a revised National Treatment Guideline for malaria. Pharmacists (96.7%) were more likely to have heard of ACTs (p= 0.019) and would prefer to treat malaria with ACTs (p= <0.001). In conclusion, antimalarial monotherapy prescription is high in Ibadan. There is an urgent need for a well structured training programme that will update community drug providers' knowledge and competence required to treat malaria correctly using ACTs. This will contribute to the ongoing efforts to control of malaria and prolong the clinical useful life of available ACTs.

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FTY720 TREATMENT DURING EXPERIMENTAL MALARIA: POTENTIAL ADJUNCTIVE THERAPY FOR CEREBRAL MALARIA?

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FTY720 is a sphingosine-1-phosphate (S1P) agonist. S1P is a tightly regulated signaling sphingolipid which binds a number of different receptors expressed on various cell types, resulting in receptor internalisation followed by later recycling. S1P binding has been implicated in cell migration, endothelial function, apoptosis and inflammation. FTY720 differs from S1P by inducing prolonged receptor internalisation, thereby extending the effect of S1P receptor signaling. To date, FTY720 has been shown to reduce lymphocyte migration and endothelial activation in models of autoimmunity. Based on the hypothesis that increased inflammatory lymphocyte trafficking to the cerebral vasculature and increased endothelial activation and dysfunction are key features of human cerebral malaria (CM), we assessed the potential of FTY720 as treatment for experimental CM in the *Plasmodium berghei* ANKA (PbA) model. C57BL/6 mice treated daily with FTY720 starting one day prior to PbA infection showed significantly improved survival on day 15 compared to non-treated animals who all succumbed from cerebral symptoms within 10 days (Log Rank Test, p<0.01). Circulating as well as splenic lymphocytes were decreased in the drug-treated mice. On day 5 of infection, we also observed a decrease in the inflammatory markers IFN-γ and TNF (p<0.05) in the FTY720-treated group. The impact of FTY720 treatment on endothelium function was examined using the vWF : ang1 ratio, two markers of endothelial activation and integrity. In uninfected mice, vWF levels are low and ang1 levels are high resulting in a low ratio, whereas the opposite is true in PbA-infected mice. FTY720-treated mice showed significantly decreased endothelial activation as reflected by a decreased ratio compared to non-treated mice. These data indicate that FTY720 improves survival and maintains endothelial integrity in the PbA model of CM and suggest a potential role for this class of compounds as adjunctive therapy in treating CM. However, further research is required to assess its possible use in human malaria.

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MALARIA DISCIPLINE: A COMPARATIVE STUDY OF AMERICAN AND AUSTRALIAN TROOPS IN SOUTHEAST ASIA, 1967-1968

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Malaria has influenced the outcome of numerous military campaigns from ancient times through the 20th century. Improved understanding of disease aetiology, transmission, and treatment have helped to reduce the impact of the disease on effective troop-strength. Late 19th and early 20th century researchers confirmed that appropriate mosquito-vector control, personal protective measures (*i.e.,* 'PPM' such as netting, insect repellents, *etc*.) and chemotherapeutic agents offered potentially effective prevention and treatment methods, especially when utilised in a multi-pronged strategy of malaria discipline. Commanders such as Field Marshall William Slim learned to apply this model effectively to operational theatres. The primary objective of this strategy was to bolster combat effectiveness through malaria prevention. Failing strategies of mosquito-vector control (MVC) and personal protective measures (PPM), medical officers utilised chemotherapeutic prophylaxes and treatments to expedite the return of troops to full duty. As mosquito-vector control (MVC) and personal protective measures (PPM) frequently proved impractical during highly mobile operations, commando raids, *etc*., chemoprophylaxis appeared to represent the most efficient strategy of 'malaria discipline' within the context of military operations. The number of United States and Australian military personnel in Vietnam escalated_officially and unofficially--after the withdrawal of the French in mid-1954. The Geneva Accords of mid-1954 partitioned Indochina at the 'Demilitarised Zone' (DMZ), the 17th parallel. By the height of the 'The Second Indochina War' in the late 1960s, the United States and Australia had committed hundreds of thousands of ground troops to Southeast Asia. Contemporary comparative data suggest that, despite various measures to enforce malaria discipline among Australian and United States troops during the Vietnam War, yielded inconsistent results. Such findings prove especially ironic in light of the potential consequences_detention in Southeast Asia for wilful disobedience--that accompanied lax malaria discipline. Disciplinary action, malaria, or a delayed return home seemed to have widely variable on malaria control objectives.

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RANDOMIZED, PROSPECTIVE, THREE-ARM STUDY OF THE AUDITORY FUNCTION FOLLOWING ANTIMALARIAL TREATMENT IN PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The observation that artemisinin derivatives adversely affect the auditory pathways in the brainstem in animal models stimulated a number of studies in humans. The studies in man have mainly been retrospective, only focused on pure-tone threshold data to determine the integrity of the auditory pathway, and did not employ adequate controls. This triggered the current study which assessed the auditory safety of artemether-lumefantrine (A-L) in comparison with other antimalarial treatments in patients with acute, uncomplicated *P. falciparum* malaria. Auditory brainstem response (ABR) was used to detect damage to auditory brainstem pathways. In this prospective, open-label trial, performed on the Colombian Pacific coast, an area where *P. falciparum* malaria is endemic, patients aged 12 years or older were randomly assigned in a 3:1:1 ratio to either A-L (N=159), atovaquone-proguanil (N=53) or artesunate-

mefloquine (N=53). Study participants entered a treatment phase of 3 days followed by a 39-day follow-up period. The presence of auditory changes compared to baseline was investigated at days 3 (1 hour after last dose of study medication) 7, 28 and 42 following treatment initiation. At each of these days, audiometric tests (e.g. tympanometry, pure-tone air conduction thresholds) and ABR measurements were performed under standardized conditions by trained audiology technicians. ABR testing was done using a validated stationary auditory evoked potential system. Results from ABR analysis were reviewed by a qualified clinician unaware of the patient allocation. The primary assessment was ABR at day 7. An increase in ABR Wave III latency (in either or both ears) of greater than 0.30 msec was to be classified as auditory nerve abnormality. A 15% or higher incidence rate of patients showing abnormal auditory function was considered clinically relevant. Final results will be presented and the clinical relevance of potential findings will be discussed.

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COHORT STUDY IN ZAMBIA EVALUATING THE SAFETY OF ARTEMETHER-LUMEFANTRINE (AL; COARTEM®) AND SULFADOXINE-PYRIMETHAMINE (SP) IN PREGNANT WOMEN WITH SYMPTOMATIC MALARIA

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Safety data on artemisinins in pregnancy are limited. A prospective cohort study was conducted in Zambia to compare the safety of AL and SP in pregnant women who received these drugs to treat symptomatic malaria. The primary objective was to evaluate the perinatal mortality rate (i.e. stillbirth or neonatal death </=7 days after birth). Secondary objectives included gestational age-adjusted birth weight. Exploratory objectives included the assessment of spontaneous abortions (</=28 weeks gestation); preterm deliveries (<37 completed weeks); neonatal mortality (</=28 days after birth); maternal mortality; birth defects; neurodevelopmental deficits in infants. Post-delivery follow-up was </=6 weeks for mothers and </=12 months for live newborns. Data from 1001 pregnant women (AL n=495; SP n=506) and 933 newborns (AL n=466; SP n=467) were analyzed. There were no clinically relevant differences in rates of perinatal mortality (AL 4.2%; SP 5.0% of newborns/stillbirths), neonatal mortality (both groups 2.3%), stillbirths (AL 1.9%; SP 2.7%), preterm deliveries (AL 14.1%; SP 17.4% of fetuses) or gestational age-adjusted low birth weight (AL 9.0%; SP 7.7% of newborns). Seven women in the AL and 5 in the SP group had spontaneous abortions. Including umbilical hernia, which is a common defect in this population (AL 4.7%; SP 2.7%), birth defect rates were 6.5% for AL and 4.1% for SP. Two infants (AL) had chromosomal aberrations. Of 6 maternal deaths (AL 1; SP 5), 3 (SP) were due to comorbid infections. Besides pregnancy related events, the most common adverse events were infections (AL 12.3%; SP 13.0%), mainly malaria (AL 3.4%; SP 6.7%) and syphilis (AL 4.8%; SP 4.0%). At one site where the Shoklo infant neurological development (ND) test was performed, there was no difference between exposure groups at 12 months of age (total score: [mean {SD}] AL: n=288, 98.0 {6.5}; SP: n=224, 97.0 {8.2}). These data suggest that exposure to AL compared to SP in pregnancy is not associated with particular safety risks in terms of perinatal mortality, malformations or infant ND.

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PHARMACOVIGILANCE OF INTERMITTENT PREVENTIVE TREATMENT IN INFANTS WITH SULFADOXINE-PYRIMETHAMINE

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Intermittent preventive treatment in infants (IPTi) using sulfadoxine-pyrimethamine (SP) administered with DPT/OPV and measles vaccines is a promising new malaria control strategy. In 2007, UNICEF launched an IPTi implementation study in 20 districts in 6 African countries. A pharmacovigilance (PV) study was conducted in two countries to estimate the incidence of adverse events (AEs) following SP-IPTi administration; and efforts to strengthen spontaneous PV reporting was evaluated in all 6 countries. Cohort event monitoring (CEM) was performed in 5 districts in Ghana and Madagascar for 7-8 months, where health staff/ volunteers made follow up home visits 7-10 days after SP administration to obtain information on AEs. Activities aimed at strengthening training and supervision for PV spontaneous reporting were implemented in 6 countries. Of the 23,998 (28% of eligibles) successfully followed after ≥1 SP doses, only 67 where followed after all three doses. No cases of Stevens-Johnson syndrome (SJS) were identified (95% upper confidence limit 1/8,000 infants). The rate of moderate AEs probably linked to immunization/IPTi was 1.75/1,000 doses. All AEs observed are also commonly found after vaccination, except for diarrhea. In the passive surveillance, where half-million doses of SP were given to 217,392 infants, there was no spontaneous reporting of mild AEs, and moderate/severe cases reported were <1% of those detected by active follow up. There was one serious AE, but it was not SJS. No cases of SJS, were identified, though few children were followed up for all doses, and cases could have occurred after follow-up or in the children not followed. For this reason, a retrospective review of hospitalizations in the CEM study area has been initiated to rule out additional SAEs. Passive surveillance may be insufficient to detect even serious side effects, and alternatives such as active hospital-based surveillance for the detection of severe events should be considered.

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GENERATION AND CHARACTERIZATION OF A 5K ANTIMALARIAL COMPOUND COLLECTION

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The availability of large compound libraries and the ability to screen them in an automated fashion is largely restricted to the pharmaceutical industry, yet is a resource with great potential in the field of drug discovery for neglected diseases. We describe here the results of screening 650,000 compounds for their activity in inhibiting proliferative growth of the human pathogen *Plasmodium falciparum*, the plasmodium species responsible for the majority of deaths due to malaria. We have generated a collection of ~5K antimalarial hit compounds, annotated with antimalarial activity against parasite proliferation in *in vitro* culture, as well as for potential cytotoxicity in the mammalian hepatocellular carcinoma cell line Huh7. In addition we have assessed this collection for rapidity of activity, synergy with currently marketed drugs, and activity in a variety of *P. falciparum* target-based biochemical assays (see ASTMH abstract "Linking anti-*Plasmodium* compounds to their protein targets via high-throughput enzyme activity assays" by G. Crowther *et al.).* Finally, we have also screened this antimalarial compound collection for cross-reactivity to other parasites including *Leishmania donovani* (leishmaniasis) and *Trypanosoma brucei* (trypanosomiasis). All compounds screened were

purchased from vendors, are non-proprietary and most remain available for purchase. This resource provides the malaria research community with access to an annotated set of compounds active against *P. falciparum* which can be used to test the candidacy of potential drug targets, and which have the potential to serve as starting points for new medicines.

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PHARMACOKINETICS OF CHLOROQUINE IN A MURINE MALARIA MODEL

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Chloroquine (CQ) has a limited clinical role in malaria treatment, but it remains the standard comparator drug for pre-clinical studies of antimalarial drugs. As pharmacokinetic data for CQ in mice are limited, evaluating combination treatments and pharmacokinetic-pharmacodynamic relationships in murine models is problematic. Hence, we have conducted a detailed investigation of the pharmacokinetics of single and multiple-dose CQ in the murine malaria model. Male Swiss mice (n = 125; mean weight 32 ± 3 g) with a 2-5% *P. berghei* parasitaemia and a parallel group of uninfected, healthy mice (125; 34 ± 3 g) were given a single dose of 50 mg/kg CQ by i.p. injection. Blood was harvested from groups of 5 mice from each group at regular intervals over 7 days and the plasma was analysed by HPLC for CQ and desethyl-CQ concentrations. In the multiple dose study, malaria-infected (125; 29 ± 3 g) and control mice (125; 31 ± 3 g) received 50 mg/kg/day CQ for 5 days. Pharmacokinetic parameters were determined using non-compartmental analysis, except the rate constant for desethyl-CQ formation and $t_{½}\alpha$ and $t_{½}\beta$ for CQ, which were estimated using a two-compartment model. In the single-dose study, the elimination $t_{½}$, CL and V for CQ were 47 hr, 9.9 L/hr/kg and 667 L/kg in healthy mice and 99 hr, 7.8 L/hr/kg and 1122 L/kg in malaria-infected mice. The $t_{½}\alpha$ and $t_{½}\beta$ for CQ were 3.3 and 53 hr in healthy mice and 4.7 and 163 hr in malaria-infected mice. The estimated formation $t_{½}$ (1.5-3 hr) of desethyl-CQ suggests that the metabolite clearance is formation rate-dependant. Pharmacokinetic parameters in the multiple-dose study were similar. The median survival time of malaria-infected mice given 50 mg/kg single dose CQ was 8 days, whilst the multiple-dose mice survived until the 30-day study end-point, with parasitaemia <1%. We conclude that CQ exhibits biphasic elimination in the murine malaria treatment model and the long elimination $t_{½}$ of CQ should be considered when interpreting data from animal studies.

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A STUDY OF INTERMITTENT PREVENTIVE TREATMENT AND HOME BASED MANAGEMENT OF MALARIA IN A RURAL AREA OF THE GAMBIA

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Malaria remains an important cause of mortality and morbidity among childre and pregnant women. The global malaria control strategies include prompt treatment with an effective antimalarial drug, vector control using ITNs or indoor residual spraying (IRS) and intermittent preventive treatment (IPT). However, individually these interventions provide only imperfect protection. Thus, there is a need to investigate whether additional control measures provide added benefit in reducing mortality and morbidity. During the 2008 malaria transmission season, 1245 children under 5 years of age were randomly allocated to receive IPT or placebo from village health workers (VHWs) based in primary health care villages. Treatment with a single dose of sulfadoxine /pyrimethamine plus three doses of amodiaquine or placebo were given to all study subjects at monthly intervals on three occasions during the peak malaria transmission season (September, October, and November). In addition, VHWs were trained to administer treatment with Coartem to children if they develop symptoms compatible with malaria during the malaria transmission season. The primary end point was incidence of clinical attacks of malaria detected by passive case detection during the study. Results of the trial will be presented.

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A TWO-GENERATION STUDY OF THE REPRODUCTIVE TOXICITY OF PIPERAQUINE IN MICE

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Antimalarial treatment options in pregnancy are compromised by inadequate safety data for newer drugs and widespread parasite resistance to conventional agents. Piperaquine (PQ) and dihydroartemisinin may be a promising combination for the management of malaria in pregnancy, due to proven efficacy in 3-day malaria treatment regimens. In humans administered PQ phosphate (PQP) at total doses up to 60 mg/kg over 2-3 days, the most common adverse effects are leucopenia, elevated serum albumin, elevated ALT and transient, mild prolongation of the QT interval. In a recent murine study, we found that PQP (1,500-3,000 mg/kg over 5 days) caused mildly abnormal white cell counts, elevated ALT and low serum albumin. Liver and kidney histopathology revealed minor inflammatory effects and occasional evidence of renal toxicity, respectively. As there is a paucity of reproductive toxicity data for PQ, we have investigated the effects of PQ on pregnant mice (F0) and two generations of their offspring (F1 and F2) using a design consistent with the requirements of ICH Guideline S5. PQP (0-300 mg/kg/day) was given to pregnant Swiss mice from gestational days 14-18. Two F1 pups from each litter were mated within matching dose groups at maturity (8 weeks). Remaining F1 and all F2 mice were euthanized at 4 weeks for determination of biochemical and haematological indices, plasma PQ concentrations and assessment of liver and kidney histopathology. There were no significant differences in growth rates, nor any dose-related developmental abnormalities observed in F1 and F2 mice. Leucocytes were mildly elevated and serum albumin was reduced in the highest dose group (300 mg/kg/day PQP). Plasma PQ concentrations in F1 mice were approximately 10% of maternal levels and PQ was detected at 8 weeks in F1 mice from dams that received 100 or 300 mg/kg/day PQP. Our reproductive toxicity study of PQ in mice showed no significant toxicity in two generations of offspring but small changes in leucocyte counts and serum albumin were observed. We conclude that monitoring women and their infants for known adverse effects is recommended when PQ is used in pregnancy.

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PHYSIOLOGICAL HIGH THROUGHPUT B-HEMATIN GROWTH ASSAY

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We have developed a simple high throughput assay for testing hemozoin crystal growth inhibition under physiologic conditions. The assay utilizes the differential solubility of crystalline and non-crystalline forms of FPIX in 2.5% (w/v) SDS and alkaline bicarbonate buffer. Notably, the optimized assay incorporates the use of a lipid catalyst, physiologic temperature, and physiological levels of heme and other reagents. We find that catalysis by monoacylglycerols is very similar to phosphatidylcholine and other lipids at 37°C. The assay requires no hemozoin purification steps and utilizes inexpensive, non-hazardous reagents. Using known antimalarials and a rationally designed set of CQ analogs incorporating mono-, di- and tribasic derivatives, we test for possible correlations between *in vitro* hemozoin

inhibition and *in vivo* antiplasmodial activities under various conditions. We find that, when corrected for variable levels of vacuolar accumulation, there is no obvious correlation between hemozoin inhibition and parasite growth inhibition for CQ analogues.

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ABSOLUTE BIOAVAILABILITY OF *CIS*-MIRINCAMYCIN AND *TRANS*-MIRINCAMYCIN IN HEALTHY RHESUS MONKEYS, AND *EX-VIVO* ANTIMALARIAL ACTIVITY AGAINST *PLASMODIUM FALCIPARUM*

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Mirincamycin was found to have radical curative activity in a relapsing primate model previously, but pharmacokinetic-pharmacodynamic relationships were not well described due to analytical methods limitations. We studied the absolute oral bioavailability (F) and *ex-vivo* antimalarial activity against *P. falciparum* of *cis*-mirincamycin (c-MC) and *trans*-mirincamycin (t-MC) in healthy rhesus monkeys. Four groups of monkeys received c-MC or t-MC, at a dose of 4mg/kg IV or 20mg/kg PO, compared to vehicle only. Plasma samples were collected at 0, 5, 15, 30, 60 min, 2, 4, 6, 8, 12, 24, 48 and 168 hours, and analyzed for pharmacokinetic parameters and *ex-vivo* antimalarial activity against a W2 clone of *P. falciparum*. c-MC had an absolute oral bioavailability of 13.6% - slightly higher than t-MC (11.7%). $AUC_{0\text{-}48\ h}$ of c-MC was somewhat higher than t-MC after intravenous dosing (4597±666 ug.h/L versus 3998±1404 ug.h/L, p=0.05), but not with oral dosing. Terminal elimination half-life was higher for oral c-MC compared to t-MC (15.4 ± 2.13 h versus 9.43 ± 0.28 h, p=0.05), but there was no difference by the IV route. Against W2 clones of *P. falciparum*, c-MC had slightly greater activity in an *ex vivo* model than t-MC, but was not statistically significant after normalization for AUC. At peak oral dose concentrations (Tmax = 2 hrs), both isomers had activity equivalent to ~25 nM of dihydroartemisinin (DHA). A higher ratio of antimalarial activity to AUC after oral compared to intravenous dosing was found for the first 90 minutes with both isomers. Adverse events for both isomers after single dose by both routes included loose stools and modest elevations in serum aspartate transaminase (AST). In conclusion, there were not significant differences between single dose c-MC and t-MC in PK or PD parameters by the IV or oral route in non-human primates. Higher ratios of *ex vivo* activity to concentration in the oral dose groups for the first 90 minutes suggests first pass metabolism with formation of an active metabolite. Both isomers will be advanced for further evaluation in a relapsing P. cynomolgi primate model.

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INDUSTRIAL CHICORY AS PRODUCTION PLATFORM FOR ARTEMISININ

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Artemisinin is a main active ingredient of the most potent anti-malarial drugs currently available, but also displays activity against a range of other diseases such as for example several forms of cancer. Because of its superb anti-malarial activity and the development of new applications the world demand of artemisinin is rapidly increasing. However, the supply of artemisinin is troublesome as total chemical synthesis is economically not feasible and the only plant species known to produce artemisinin, *Artemisia annua L.*, contains only low amounts of this compound (up to about 0.8% of dry weight) and does not produce a suitable amount of biomass per hectare. To improve artemisinin availability, the production in microorganisms is one option, as reported previously, even so the production in alternative highproducing industrial plant species another one. Our aim is to identify a production system with higher yield per ton plant material, and a production system which allows growing more tons per hectare. Research on the biosynthesis of sesquiterpene lactones in chicory led to the discovery that a chicory cytochrome P450 enzyme *in vitro* also very efficiently oxidises amorpha-4,11-diene to artemisininic alcohol, as reported previously. Therefore we are establishing the suitability of industrial chicory for the production of artemisinin precursors. This will be achieved using a combination of molecular biology, altered subcellular enzyme compartmentation, biosynthesis and *in vivo* bio-transformation. Chicory is transformed with the *A. annua* amorphadiene synthase, the enzyme catalyzing the first step in artemisinin biosynthesis, as reported previously. Expression of this gene and amorphadiene formation is optimised by targeting amorphadiene synthase to different subcellular compartments with and without additional substrate delivering enzymes. We anticipate that the (transgenic) amorphadiene is converted by the native chicory P450 to artemisinic acid or dihydroartemisinic acid considering that sesquiterpene double bond reductase activity is also present in chicory. Before stable transformation is done, the different gene combinations are evaluated in a fast transient transformation system in chicory. Preliminary results show that the approach works and that high yields of artemisinin precursors are feasible.

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TETRAZOLIUM VIOLET INHIBITS MEROZOITE INVASION

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We have previously reported that tetrazolium salts disrupt the growth of *Plasmodium falciparum* cultures when present in nanomolar concentrations. To examine why tetrazolium salts inhibit *P. falciparum* cultures we monitored the developmental stage of synchronized parasite cultures in the presence of varying amounts of Tetrazolium Violet (TV). TV prevented intracellular development of the parasites when it was present in micromolar concentrations, however normal development was observed in cultures at concentrations of TV of less than 1 micromolar. When the treated cultures were allowed to form schizonts, rupture, and invade new erythrocytes, it was observed that extracellular merozoite forms of the parasite were present at TV concentrations above 100 nanomolar and ring forms of the parasite were absent. Cultures treated with concentrations of TV of less than 1nM subsequently successfully formed trophozoites, while cultures with large numbers of extracellular merozoites present did not. Treatment of unparasitized erythrocytes (UEs) and erythrocytes parasitized with mature-stage parasites (PEs) with 800nM TV for 3 hours, followed by the removal of the TV from the media, resulted in the inhibition of merozoite entry in cultures containing pre-treated PEs, but not UEs, suggesting that TV interacts with a parasite component only. Samples of the murine malarias *P. chabaudi chabaudi* and *P. berghei* were allowed to develop in the presence of varying concentrations of TV and the viability of the cultures were determined using SYBR Green I. It was observed in parallel assays that TV had IC_{50} values of 93 ± 19nM, 211± 23nM, and 400 ± 21nM for *P. falciparum*, *P. chabaudi* and *P. berghei* cultures, respectively. Taken together our results suggest that TV inhibits *Plasmodium* cultures by interfering with a merozoite component that is present in at least three species of *Plasmodium*.

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MONOQUINOLINE ANALOGUES HIGHLY ACTIVE AGAINST THE BLOOD STAGES OF *PLASMODIUM IN VIVO* AND *IN VITRO*

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New drugs to treat malaria must act rapidly, be highly potent against asexual blood stages, be well tolerated and must be affordable to residents of endemic regions. This was the case of chloroquine (CQ), a 4-aminoquinoline that has been long been used for the prevention and treatment of malaria. However, since 1960's *Plasmodium falciparum* resistance to this drug has spread to all regions of the world and more recently emerging resistance to chloroquine by *Plasmodium vivax* threatens the health of hundreds of millions of people. Despite the emergence of CQ resistance, synthetic quinoline derivatives remain a validated lead class for new drug discovery, especially if they are effective against CQ resistant strains of malaria. In this study, we investigated the activity of two monoquinoline derivatives (Ro 61-0732 and Ro 47-8505) and we found these compounds are active against *Plasmodium in vivo* and *in vitro*. All the *P. falciparum* parasites tested show sensitivity *in vitro* to Ro 61-0732 and Ro 47-8505, including the chloroquine resistant reference clone W2 and several multidrug resistant parasites recently isolated from Thailand and Cambodia (IC50s ranging from 0.97 to 13.51 ng/ml). In addition, these drugs had curative activity in Balb/c mice infected with *P. berghei* after oral administration. In fact, 80 mg/kg of Ro 61-0732 and Ro 47-8505 were sufficient to cure all the mice tested without signs of toxicity. Parasitemia cleared rapidly following 3 days of treatment in a modified Thompson Test model and no parasites recrudesced during the 30 day experiment. Additional studies are underway to examine the potential use of the monoquinolines in combination therapy with other antimalarials and potentiating compounds. Our findings suggest that these novel monoquinolines should be considered for development as potent antimalarials to treat multiple drug resistant *P. falciparum* and *P. vivax.*

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ANTI-*PLASMODIUM* ACTIVITY OF IMIDAZOLIUM AND TRIAZOLIUM SALTS

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We have previously reported that sulfated cyclodextrins inhibit the invasion of *Plasmodium* merozoites by interacting with receptors present on the surface of erythrocytes. The observation that tetrazolium salts formed stable complexes with the inhibitory sulfated cyclodextrin compounds suggested that tetrazolium salts might have anti-*Plasmodium* activity as well. In an extensive study we determined that tetrazolium salts indeed were both potent and specific inhibitors of *Plasmodium* replication, and that they appear to interact with a component of the parasite that is both essential and conserved. The use of tetrazolium salts *in vivo* is limited by the potential reduction of the tetrazolium ring to form an inactive, neutral acyclic formazan; also, the tetrazolium ring may be photochemically unstable. To address these issues imidazolium and triazolium salts were synthesized and evaluated as *Plasmodium* inhibitors. A total of 8 novel triazolium compounds were synthesized and evaluated and were found to have therapeutic ratios ranging from 1 to >1,000. Further, a total of 22 novel imidazolium compounds were synthesized and evaluated as drug candidates. Many of the imidazolium salts were both highly potent, with active concentrations in the nanomolar range in *P. falciparum* cultures, and specific to *Plasmodium* with therapeutic ratios ranging from 2 to 250. Indeed, the new compounds have provided potential drug candidates possessing greater potency and selectivity than the original tetrazolium salts. Moreover, the results corroborate our hypothesis that an electron-deficient core is required so that the compound may thereby interact with a negatively-charged moiety on the parasite merozoite ligand; the side groups in the compound then form favorable interactions with adjacent parasite components and thereby determine both the potency and selectivity of the compound.

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SAFETY OF PYRONARIDINE/ARTESUNATE IN CLINICAL TRIALS IN PATIENTS WITH UNCOMPLICATED ACUTE *PLASMODIUM FALCIPARUM* OR *PLASMODIUM VIVAX* MALARIA: RESULTS OF AN INTEGRATED ANALYSIS

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Pyronaridine/artesunate (Pyramax®/PA) is a novel and promising artemisinin combination therapy (ACT) developed in Public-Private-Partnership between Shin Poong Pharmaceuticals, Medicines for Malaria Venture and the University of Iowa. This fixed-dose combination of pyronaridine tetraphosphate (PP) and artesunate (AS) in a 3:1 ratio, is developed as an oral treatment OD for 3 days, for uncomplicated *Plasmodium falciparum* (*Pf*) malaria and for blood stage of *P.vivax* (*Pv*) malaria in adult and pediatric patients. PA should be available from 2010 in Africa and Asia. The clinical program comprised 6 randomized controlled trials; 2 phase II and 4 phase III studies including a total of 4071 patients. Overall, a total of 2815 patients were treated with PA, including 1528 adults (≥18 years), 401 patients 12-18 years and 886 children (<12 years). Integrated analysis of efficacy (ISE) and safety (ISS) were performed on the pooled data and the outputs and conclusions regarding the ISS are discussed here. The clinical trial safety database included 2033 patients from Asia and 2038 from Africa. The integrated safety analysis showed the following: 57.2% of PA patients reported at least 1 adverse event (AE) after baseline. Overall, the rate and type of AEs were generally comparable between the various treatment groups and were of mild or moderate severity. The most frequently reported AEs (headache 10.6%, cough 5.9%, anemia 4.5%, vomiting 4.4%) were unspecific, likely to be related to the signs and symptoms of malaria. There were no deaths and few SAEs (0.6% in PA patients) and most of them were not related to PA. Overall, no changes in vital signs were observed except for a fall in heart rate immediately after treatment probably reflecting the clearance of fever. The pattern of changes seen in clinical laboratory parameters was consistent with acute malaria and its resolution after treatment. All biochemistry changes were transient and reversible and not associated with any symptomatology. PA was not associated with any increased risk of hematologic AEs. PA-treatment was not associated with an increased risk of QTc prolongation. This ISS demonstrated a good safety and tolerability profile of PA in *Pf* and *Pv* malaria in adult and children patients. PA is a promising new ACT for the treatment of acute malaria in adults and children from 5kg.

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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF GENZ-644442 ANALOGS: A NOVEL CHEMOTYPE WITH POTENT ACTIVITY *PLASMODIUM FALCIPARUM* AND *P. BERGHEI*

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Genz-644442 was identified as a "hit" from a library of ~73,000 compounds by high-throughput screening campaign performed at the Institute of Chemistry and Cell Biology (Harvard Medical School) and the Broad Institute in collaboration with Genzyme Corporation and

Malaria Medicines Ventures and later transitioned to 'Lead Identification'. Genz-644442 has IC_{50}'s of 200, 520, and 285 nM against *Plasmodium falciparum* strains 3D7, HB3, and Dd2 respectively. *In vitro* absorption, distribution, metabolism, and excretion (ADME) studies demonstrated high metabolic stability in human and rat hepatocytes, lack of human CYP inhibition, moderate lipophilicity with a Log D of 3.0, good solubility and permeability characteristics. Additionally, the compound has a good selectivity profile for parasite versus normal human cell lines (>100X IC_{50}). The compound was well-tolerated in mice when dosed over 4 days at doses up to 50 mg/kg/day. Pharmacokinetic (PK) studies in mice showed that Genz-644442 had a terminal $T_{1/2}$ of 7.45 h and plasma levels of 30X the IC_{90} when dosed intraperitoneally at 50 mg/kg. When the racemic mixture was dosed at 50 mg/kg/day for 4 days in mice infected with *P. berghei*, no parasites were detected on Day 4 post infection; however, parasites recrudesced by Day 9. In contrast, one enantiomer cured 2/5 mice (no detectable parasites by Day 28 post infection). An efficient chemical route was established to afford access to analogs to probe SAR. Synthesis of a small library of compounds coupled with the *in vivo* data allowed the program to transition to 'Late Lead.' A medicinal chemistry effort to optimize for potency and ADME properties was undertaken and has led to identification of compounds with IC_{50}'s of approximately 10 nM, while maintaining good selectivity and a favorable ADME profile. Compounds are routinely assessed for exposure and tolerated dose before progressing to the *P. berghei* model. Several compounds have been identified that when dosed PO result in reduction of parasitemia. Efforts are underway to select the best candidates for progression to preclinical and clinical development.

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A DUAL-SPECIFICITY PHOSPHOTYROSINE PHOSPHATASE OF *PLASMODIUM FALCIPARUM* AND ITS POTENTIAL AS A NOVEL ANTIMALARIAL DRUG TARGET

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Malaria, resulting from infection with parasites of the genus *Plasmodium*, is a severe disease resulting in 300-500 million cases of clinical illness and 1-3 million deaths annually. The identification of novel drug targets is critical. We identified an essential protein phosphatase (PF13_0027) expressed in all stages of development that is conserved among *Plasmodium* species. Bioinformatics analyses identified the presence of an inactive N-terminal rhodanese domain and a dual-specificity phosphotyrosine protein phosphatase II domain possessing the conserved residues required for catalytic activity. The ability to express the recombinant functional phosphatase domain in *E. coli* has presented the opportunity for further assays evaluating the phosphatase activity, substrate interactions and inhibition with known phosphatase inhibitors. PF13_0027 possesses characteristics similar to both CDC25 and MAPK phosphatases and is potentially a novel target for future therapeutic intervention. Therefore this investigation aims to determine the precise biological significance and evaluate the effectiveness of this phosphatase as a drug target.

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INHIBITORS OF FABI AS POTENTIAL ANTIMALARIAL PROPHYLACTIC AGENTS

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Our recent studies with *Plasmodium* parasites have shown that FabI (enoyl ACP reductase), a key enzyme in type II fatty acid biosynthesis, is not required for asexual blood stage growth but is essential for the maturation of the preceding liver stage, as reported previously. Therefore we hypothesized that compounds targeting FabI might be prophylactic against pre-erythrocytic stage infection. We investigated the suitability of triclosan and two compounds synthesized by GlaxoSmithKline (GSK) for their ability to block *in vitro* liver stage parasite development. GSK compounds 6A and 3A were selected based on biochemical assays that showed potent and specific *in vitro* inhibition of PfFabI activity. Results indicate that both compounds alone were able to inhibit the formation of exo-erythrocytic forms (EEFs) at 1.0 and 3.0 μM concentrations. A similar level of potency was observed with the liver stage drug primaquine tested at these concentrations. Triclosan inhibited the formation of EEFs with an IC_{50} value of 24 μM. Inhibitory activity of these compounds was confirmed in a time course analysis of liver stage parasite development. Triclosan also showed prophylactic activity against *P. yoelii* in a three-day suppressive test in mice. In this experiment, mice were subcutaneously administered 128 or 256 mg/kg triclosan and inoculated intravenously with sporozoites at the time of the second dose. Results showed a delay to 2% patency of at least 3 days in the triclosan-treated mice as compared to a control group treated with vehicle. Peak parasitemias in the triclosan groups were also markedly lower than in the controls. Using drug-resistant mutant forms of FabI, determined biochemically, we are currently assessing whether this antiparasitic prophylactic activity is mediated through inhibition of this fatty acid synthesis enzyme.

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THE USE OF TRANSGENIC *PLASMODIUM FALCIPARUM* IN THE EVALUATION OF IN VITRO ANTIPLASMODIAL ACTIVITY OF ANTIMALARIAL DRUGS AND METHOTREXATE

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This study reports the development of an alternative, simple, robust method, using transgenic parasites expressing a reporter gene (green fluorescent protein), and the detection and screening of this reporter gene by fluorescent activated cell sorting (FACS). The study also assessed the potential value of Methotrexate as an antimalarial agent. The novel method was compared to the much adaptable microscopic counting, to assess drug susceptibility in a standard *in vitro* assay. The transmembrane 4 (Tm4) clone of *Plasmodium falciparum* in continuous culture was transfected and used for the drug screening. Both assay methods were used to determine the inhibitory concentration that resulted in a 50% reduction in parasite viability. Nonlinear regression was used to estimate the 50% inhibitory concentration (IC_{50}) and its confidence intervals. The two methods were observed to have similar reproducibilities, but the FACS method demonstrated a higher sensitivity relative to the results of radiolabelled assays. The mean IC_{50} value ± standard deviation for transfected parasites by FACS are: Chloroquine 3.41 ± 0.46nM, Pyrimethamine 24.70 ± 2.42nM, Mefloquine 8.37 ± 1.49nM, Dihydroartemisinin 0.22 ± 0.04nM and Methotrexate 29.98 ±5.96nM, while the mean IC_{50} values of the normal parasites by microscopy are 2.98 ± 0.28, 28.76 ±1.34, 10.83 ± 2.19, 0.27 ± 0.07, 33.32 ± 2.13, for Chloroquine, Pyrimethamine, Mefloquine, Dihydroartemisinin and Methotrexate respectively. There was no significant difference ($P<0.05$) between the methods for the drugs tested except for Mefloquine ($P=0.015$), chloroquine ($P= 0.187$), pyrimethamine ($P= 0.066$), dihydroartemisinin ($P= 0.064$) and methotrexate ($P= 0.075$). Methotrexate showed activity against Tm4 clone of *Plasmodium falciparum in vitro* with IC 50 value of < 40nM. The availability of this simple and highly sensitive reporter signaling assay will provide an easier method for drug susceptibility testing of malaria parasites and it may be an ideal method for high-throughput antimalarial drug screening.

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A HIGH THROUGHPUT SCREEN TO IDENTIFY APICOPLAST-TARGETING ANTIMALARIALS

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Plasmodium falciparum has successfully parasitized a large portion of the world's population and has resisted numerous attempts at antimalarial control. This Apicomplexan parasite possesses an unusual organelle, termed the apicoplast, which is believed to derive from a secondary endosymbiotic event whereby a protozoan ancestor engulfed a red alga, containing a photosynthetic plastid of cyanobacterial origin. The apicoplast has been implicated in various processes including the synthesis of fatty acids, heme, and isoprenoids. While the exact role of the apicoplast remains enigmatic, it is clearly essential, as antibiotics that interfere with apicoplast translation, such as azithromycin, kill *P. falciparum in vitro* and *in vivo*. This apicoplast inhibition is unusual in that it manifests itself in the progeny of drug-treated parasites, a phenotype known as "delayed death." To help elucidate the function of the apicoplast, and to identify potential therapeutic agents, we have developed a luciferase-based quantitative high throughput screen to search for antimalarial compounds that appear to target the apicoplast based on their delayed death phenotype. We here report on our results generated from a small library of bioactive compounds that was screened to demonstrate the feasibility of our protocol. Our findings indicate that antimalarial compounds exhibiting a delayed death phenotype are enriched in macrolide antibiotics and drugs that interfere with lipid metabolism. Certain channel blockers and receptor binding compounds, such as serotonin agonists, also demonstrated an apparent anti-apicoplast activity. We are currently validating our hits to confirm that the apicoplast is the target of those drugs exhibiting a delayed death phenotype.

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HYPOTHESES OF DRUG RESPONSE DYNAMICS TO EXPLAIN RECRUDESCENCE IN ARTESUNATE RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

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Artemisinin combination therapies are recommended first line therapy for *Plasmodium falciparum* malaria globally. Artemisinin resistant *P. falciparum* has recently been identified for the first time in Pailin, Western Cambodia. If it spreads elsewhere, this resistance has the potential to become a major threat to malaria control efforts worldwide. Although clinically characterized as prolonged parasite clearance times (PCT), the biological substrate of this resistance is unknown. In order to gain a more detailed understanding of artemisinin resistance, parasite clearance data from Western Cambodia were compared with data from North-Western Thailand, where the clinical response to the artemisisins is still adequate. Because clearance times are partially determined by the initial parasite load, we propose a new measure to quantify drug efficacy at the individual and population levels, independent of initial parasitaemia. Initial parasite clearance rates did not differ between patients with or without a recrudescent infection during 63 days follow-up, but were significantly faster in North Western Thailand. Most infections in Western Cambodia appeared to have a similar artemisinin- resistant phenotype, defined by their slower clearance rate, and there was no clear evidence of a highly artemisinin-resistant subgroup. Using this data and the behaviour of the resistant phenotype in in-vitro test systems, we have modeled several hypotheses to explain parasite behaviour during the sub-microscopic period before recrudescence.

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ANALYSIS OF CHLOROQUINE TRANSPORT BY PURIFIED, RECONSTITUTED PFCRT

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Detailed kinetic analysis of transmembranous chloroquine (CQ) transport is needed to further understand the mechanisms behind chloroquine resistance in *Plasmodium falciparum* conferred by mutations in *P. falciparum* Chloroquine Resistance Transporter (PfCRT). Various investigators have proposed that PfCRT may function either as a channel or an active transporter. Using a novel probe (NBD-CQ), wherein the fluorescent NBD tag is attached to a terminal CQ ethyl group, purified isoforms of PfCRT reconstituted into proteoliposomes (PL), and a novel assay involving extra - PL quenching with dithionite, efflux was analyzed under a variety of conditions. Turnover numbers derived from initial rates of efflux show that the outward movement of CQ is membrane potential dependent. These data strongly support our previously reported proposal that PfCRT catalyzes facilitative diffusion of charged CQ.

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RESISTANCE TO CHLOROQUINE CYTOTOXICITY IS NOT THE SAME AS RESISTANCE TO CHLOROQUINE CYTOSTASIS

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Chloroquine resistance in (CQR) *Plasmodium falciparum* parasites is usually characterized in the laboratory by reduced CQ accumulation and an almost 10-fold higher CQ IC_{50} relative to CQ sensitive (CQS) parasites. However, accumulation of drug is almost always studied without perfusion using low (nanomolar) concentrations of radiolabeled CQ and IC_{50} is almost always calculated via cytostasis (growth inhibition) assays. This could be misleading, since in the body, micromolar CQ plasma levels kill parasites, they do not slow their growth, and CQR parasites are as resistant to CQ toxicity as they are to CQ cytostatic effects, as reported previously. In addition, we now know that continuous vs. bolus drug dosing results in different parasite stage-specific effects. We have therefore quantified CQ cytotoxicity in CQS vs. CQR parasites using a wide range of external CQ concentrations, and have measured drug accumulation under similar conditions. Results suggest that the mechanism of CQ cytotoxicity resistance is not the same as the mechanism of CQ cytostasis resistance.

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EFFICACY OF NON-CONTROLLED INTERMITTENT PREVENTIVE TREATMENT (IPTP) *VERSUS* CONTROLLED IPTP IN PREGNANT (IPTP) WOMEN IN CÔTE D'IVOIRE

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Malaria in pregnancy is one of the major causes of maternal morbidity and adverse birth outcomes. Intermittent Preventive Treatment with sulfadoxine/pyrimethamine (SP) given routinely to pregnant women irrespective of the presence of peripheral malaria parasites is part of the World Health Organization (WHO) strategy to reduce the risk of malaria in pregnancy and its consequences on the mother and her baby (IPTp). The coverage of the intervention is still low in Côte d'Ivoire. The study was performed in Côte d'Ivoire from January 2008 to March 2009.Two doses of SP, one on the second trimester (after quickening) and one on the